From:	Sack, Chris A
To:	McSeveney, Megan
Cc:	Robin, Lauren P; South, Paul
Subject:	RE: URGENT Media Inquiry - Glyphosate - CBS Evening News- 5:00 pm today deadline
Date:	Friday, January 27, 2017 4:21:00 PM

Megan, we have no updates at this time. The proposed response is still current.

Have a wonderful weekend,

Chris

Ph: 240-402-2464

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

Hi Chris,

Can you take a quick look at the response below.

Thanks, Paul

From: McSeveney, Megan
Sent: Friday, January 27, 2017 3:36 PM
To: Robin, Lauren P; South, Paul; Garrett, Ulysses; ORA PAESpress
Cc: FVM TRADE PRESS; Naum, Marianna; Eisenman, Theresa; Cassell, Peter; Shapinsky, David
Subject: URGENT Media Inquiry - Glyphosate - CBS Evening News- 5:00 pm today deadline

Hi all, Below we have a new inquiry on glyphosate from CBS news. My proposed response is based on the latest update I had from folks on January 13 – have there been any new updates? **If nothing else- can we at a MINIMUM confirm that testing has begun, but no results are available?**

Also, the information below has been previously cleared for other inquiries, but as always I wanted to make sure there have been no new updates and check with you all on the nuances. Thank you! M7

Media Inquiry

Reporter: Ruth Coxeter

Outlet: CBS Evening news

Deadline: ASAP, 1/27/2017 - **5:00pm hard deadline**

Subject: Glyphosate

Additional Information:

Is there an update on glyphosate food residue testing? I'm working on a piece on today's

Monsanto/California hearing.

"The FDA said in February that it would begin testing for glyphosate residue in food in the U.S. The results aren't yet available"

https://www.bloomberg.com/news/articles/2016-03-10/monsanto-s-roundup-could-get-whacked-by-european-regulators

Proposed response:

(b) (5)

[redacted]

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.
Sent: Wednesday, January 04, 2017 1:53 PM
To: Chang, Eugene
Cc: Sack, Chris A
Subject: Glyphosate Method

Eugene,

I'm still having great results from the 4 mM TBS-OH (pH 2.8) Mobile Phase. I'm using straight acetonitrile for mobile phase B. I added a 4 minute equilibration period to get the column back to an initial ion pair state after the ACN finish of the previous run.
I'm running Narong's 7 point calibration curve before and after a sample batch to check for ruggedness and column drifting and I have not seen any problems. The curve points lay on top of each other very well. I spiked a batch of broccoli at 0.002 ug/g and could detect the glyphosate pretty well but I did need to manually integrate some of the peaks . I used broccoli because it's the only thing I have on hand that does not have glyphosate in it. I have brought wheat crackers, granola cereal, and corn meal from home and there's a fair amount in all of them.

The curtain plate is staying pretty clean too. A bit worse than the pesticide method for sure but very usable. I am setting the divert valve to exclude as much as possible.

I have not received the N-acetyl glyphosate yet so I am concerned that it works as well. It should arrive soon.

I thought I would let you know how things are going.

Regards,

Richard Thompson
Chemist
US FDA
Arkansas Regional Laboratory
Pesticides Laboratory
Tel 870-543-4054
Richard.thompson@fda.hhs.gov


FDA
U.S. FOOD & DRUG
ADMINISTRATION
OFFICE OF REGULATORY AFFAIRS

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; Cromer, Michele
Subject: RE: Revised Minutes for PesTAG meeting Dec 14, 2016
Date: Monday, January 09, 2017 1:04:00 PM

Hey everyone,

Just wanted to touch base to see how you are progressing with the glyphosate method implementation. Please let me know if your lab

1. purchased the supplies and reagents
2. attempted the LCMS method
3. generated recoveries using whole method
4. received the collaboration matrices
    a. carrots from KAN
    b. avocado from ARL

Thanks,

Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Friday, December 16, 2016 11:13 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Cc: Noonan, Gregory; Cromer, Michele
Subject: Revised Minutes for PesTAG meeting Dec 14, 2016

PesTAG PMC Meeting Minutes

Date: December 14, 2016

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

Agenda: Glyphosate method progress and collaboration

We really need to get this method finalized, validated and collaborated. Although there is some external pressure to resume the assignment, we are not late yet. Sack assured CFSAN-OC that the assignment would resume in early CY-17 and we can still make that timeframe.

LC-MS/MS method
Eugene updated his latest LC-MS/MS method that uses ion-pairing with reverse phase chromatography. The essential LC parameters are listed below and listed in the attached file.

	LC Parameters	**Gradient**	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	10 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±1)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.6 mL/min (4.6 mm column)	5.00	95

Inj Vol:	10 µL	6.50	95
Temp	45 °C	6.60	5
		10.00	5

MS/MS Parameters

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	4.0	Glufosinate 1	-60	-11	-66	-9
180	95	4.0	Glufosinate 2	-40	-11	-24	-5
180	85	4.0	Glufosinate 3	-60	-11	-25	-9
183	63	4.0	Glufosinate IS	-60	-11	-40	-9
168	63	5.0	Glyphosate 1	-30	-11	-28	-9
168	79	5.0	Glyphosate 2	-30	-11	-56	-9
168	150	5.0	Glyphosate 3	-30	-11	-16	-9
171	63	5.0	Glyphosate IS	-30	-11	-28	-9
210	63	6.0	N-acetyl glyphosate 1	-85	-11	-40	-13
210	124	6.0	N-acetyl glyphosate 2	-85	-11	-17	-13
210	79	6.0	N-acetyl glyphosate 3	-85	-11	-50	-13

MS Parameters

CUR	25
CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	700
Q1	UNIT
Q3	UNIT

Preparation of the mobile phase:

- For 1 liter mobile A: to make 10 mM TBAOH, add 25 ml of 0.4 M TBAOH solution to ~900 mL DI water containing 0.40 mL formic acid. Adjust pH to 2.8-3.2 using 0.1% formic acid solution. OR
- Add 1.0 mL formic acid (98%) and 3.01 grams TBA acetate in 1 L DI water; pH range between 2.8-3.0.

Eugen provided chromatograms using the current LCMS parameters for standard mixture, corn control and corn spiked at 10 ng/g (see attached).

Several people challenged Eugene's use of neat MeCN for the organic modifier, suggesting it should contain the same level of IP reagent and have same pH as the aqueous phase. Eugene defended his method saying it did not seem to be a problem; however as he uses the method to analyze matrices he will observe the stability of the LCMS response and retention.
Richard mention he tried this latest LC method and found the glyphosate was tailing. Eugene suggested replacing all metal LC tubing with PEEK tubing between the autosampler and

injection valve (see attached pic) because glyphosate can be retained on metal surfaces.

Richard alerted everyone to be sure and order the correct glyphosate isotope. He inadvertently ordered the wrong isotope using different carbon labelling and was not getting any response until he realized the mass of the isotope he ordered was actually different than that for the method.

What about others, e.g. ethephon, quats, …? Groan, this will have to wait till we get glyphosate assignment restarted.

Eugene provided a chromatogram of a 10 ng/g glyphosate in corn (attached).

Extraction Procedure

1. 5 g sample + 25 ml extraction solvent
   2g sample plus 10 ml extraction solvent for dry products
   [Extraction solvent: 50 mM Acetic acid + 10 mM EDTA]
2. Add 10 ml PE or MeCl to fatty matrices
3. Spike with isotopes @ 200 ng/g (could be included in the extraction solvent)
4. Shake @ 1000 for 10 min
5. Centrifuge at = 3000 rpm for 5 min
6. Filter aqueous extract thru HLB SPE cartridge
7. Filter for injection (could be included with SPE step)
8. Sample concentration: 0.2 g/ml

Initial Validation (LA)
Instrument:

- Evaluate linearity and range of calibration curve from 10 to 2500 ng/ml.
- Determine instrument sensitivity @ 1, 2, and 5 ng/ml – Need S/N of 10:1 for calculation ion and 3:1 for the confirmation ion
- Determine instrument response precision during validation analyses

Method

- Matrices: carrots, avocado, and corn
- Selectivity: analyze matrix blanks
- Sensitivity: analyze = 7 replicate spikes at 10 ng/g, calculate MDL per 40 CFR 136, calculate the LOQ as 3.3 x MDL
- Linearity/Accuracy: analyze 6 replicates of each matrix spiked at 50, 200, and 500 ng/g
- Range: analyze duplicate spikes at 1000 ng/g

Collaboration
We agreed to collaborate corn, carrots and avocado using the following fortification protocol.

QC	Matrix	Level	N
Control	Corn	0	1
Linearity	Corn	50	2
Linearity	Corn	200	2
Linearity	Corn	500	2
Control	Carrot	0	1
Linearity	Carrot	50	2
Linearity	Carrot	200	2
Linearity	Carrot	500	2
Control	Avocado	0	1
Linearity	Avocado	50	2
Linearity	Avocado	200	2

Linearity	Avocado	500	2
	Total Analyses per Lab		**21**

Narong has previously shipped both corn and soy with and without incurred residues to all participating ORA labs .KAN has carrots and ARL has avocados they will ship to participating labs. Each lab will receive ~ 100 g composite per matrix.

Lab	Contact	Address	Phone
PNW	Bill Cooke	22201 23rd DR SE, Bothell WA, 98021	(425) 487-5324
PSW	Eugene Chang	19701 Fairchild, Irvine, CA	(949) 608-2970
KAN	John Vonderbrink	11510 W 80th St, Lenexa, KS 66214	(913) 752-2703
ARL	Richard Thompson	3900 NCTR Road, Jefferson, AR 72079	(870) 543-4054
SRL	Narong Chamkasem	60 Eighth St NE, Atlanta, GA, 30309	(404) 253-2302
NRL	Claude Masse	158-15 Liberty Ave Jamaica NY 11433	(718) 340-7050
CFSAN	Greg Noonan	5001 Campus Drive, College Park, MD 20740	(240) 402-2250

Action items

1. Purchase reagents and supplies – all labs
    a. Phenomenex Luna C8, 150 x 2 mm, 5 µm, Phenomenex 00F-4040-B0 or Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
    b. Phenomenex guard column KrudKatcher P/N AFO-8497
    c. Glyphosate isotope
    d. Glufosinate isotope
    e. Tetrabutylammonium hydroxide titrant, 0.4 M in Water, HPLC Grade, ACROS Organics (pic attached)
    f. Tetrabutylammonium acetate, Aldrich No. 335991-10G (pic attached)
    g. N-acetyl-glyphosate, available from Toronto Research Chemicals (TRC No A178245), or Santa Cruz BioTech (SCBT No. sc-479500)
2. Ship collaboration matrices
    a. KAN ships 100 g carrots
    b. ARL ships 100 g avocados
3. LA validates method and submits validation report for review
4. All labs set up LA LC-MS/MS method on AB 5500 or 6500

Have a nice holiday,

Chris

From: Sack, Chris A [mailto:Chris.Sack@fda.hhs.gov]
Sent: Tuesday, January 10, 2017 11:49 AM
To: (b) (6)
Cc: (b) (6)
Subject: RE: Glyphosate analytical method

Hi Pamela,

I hope your holidays were full of everything good.

Do you have any experience with N-acetyl-glyphosate? We are attempting incorporate it into our procedure and finding it is quite unstable, quickly degrading to glyphosate.

Thanks and happy new year,

Chris Sack

Residue Expert
Office of Food Safety
Center for Food Safety and Applied Nutrition
US Food and Drug Administration

Phone: 240-402-2464

(b)(4) Consultation

From: Sack, Chris A [mailto:Chris.Sack@fda.hhs.gov]
Sent: Wednesday, June 15, 2016 6:43 AM
To: (b) (6)
Subject: RE: Glyphosate analytical method

Hi Pam,

Thanks for sharing your experiences. I will forward your observations to our team of analysts who are working to improve Narong's method to make it more rugged. Interesting, that you point out the problems with the chrom. It was the first problem our analysts noted about the method also. Since then, they have come up with a "gradient" using acetonitrile and the method aqueous MB to move the peaks out to about 4-5 minutes. That seems to have fixed that problem. Re the MeCl2, I know our analysts will not use it because it is chlorinated; we have dispensed with everything chlorinated in the last 10 years. So, I am glad you found another cleanup using the Phenomenex Strata-X Drug-N plate. We will look into that.

Thanks again for taking time to review the method. I am hoping to have a finalized method by end of summer so we can collaborate it in the fall.

If I invited you to join us for a telecon to discuss our glyphosate method development, would you be able/willing to accept?

Will keep in touch.

All the best,

Chris Sack
Residue Expert
Office of Food Safety
Center for Food Safety and Applied Nutrition
US Food and Drug Administration

Phone: 240-402-2464

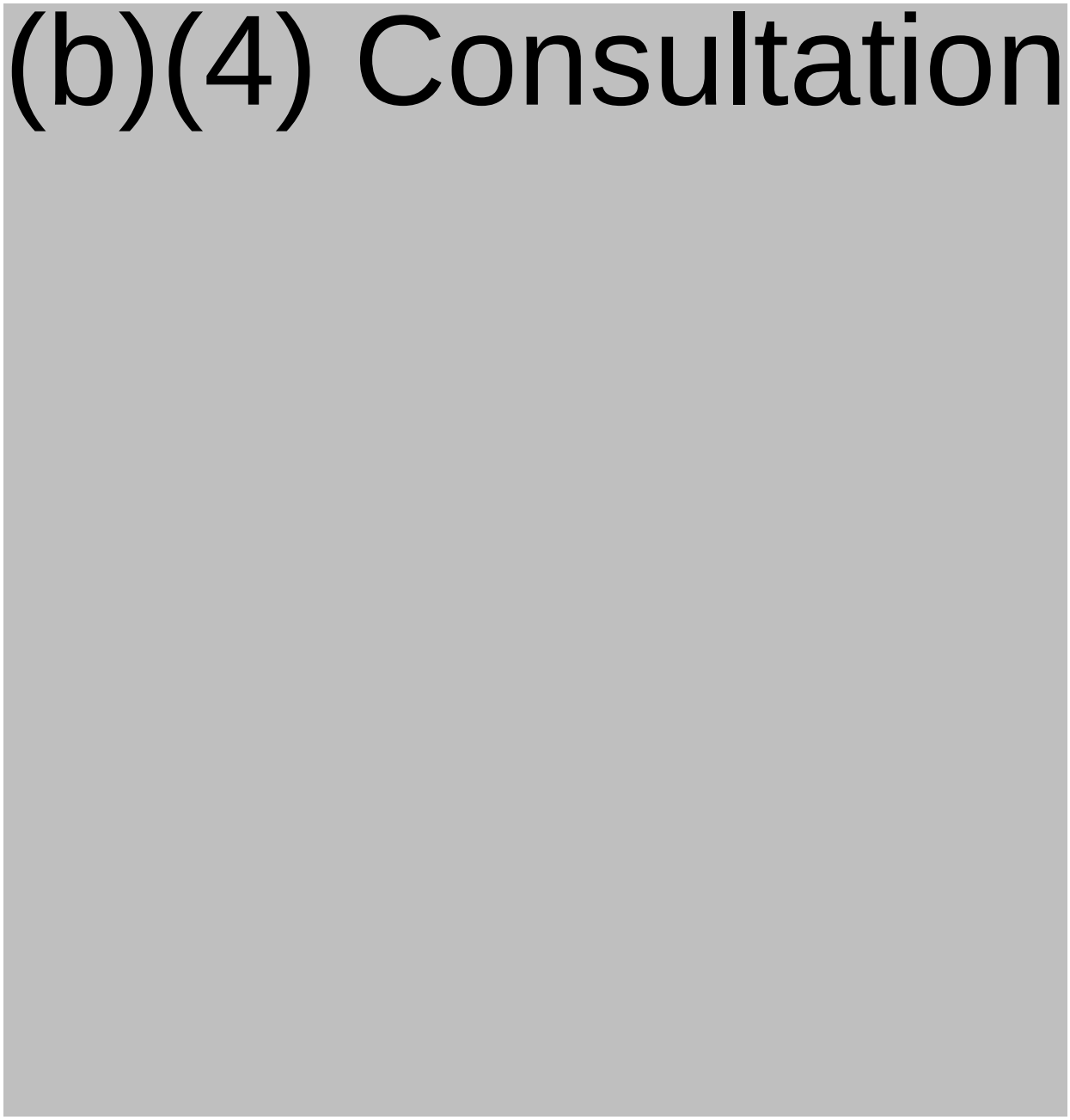
(b)(4) Consultation

From: Sack, Chris A [mailto:Chris.Sack@fda.hhs.gov]
Sent: Wednesday, June 08, 2016 7:09 AM
To: (b) (6)
Cc: (b) (6)
Subject: RE: Glyphosate analytical method

Thanks Marion!

Chris

Ph: 240-402-2464


(b)(4) Consultation

(b)(4) Consultation

From: Chang, Eugene
To: Sack, Chris A
Subject: One more step to conclude N-acetyl glyphosate
Date: Tuesday, January 10, 2017 4:45:39 PM
Attachments: 2015.11. Risk assessment glyphosate EFSA.pdf

Hi, Chris,

An EC document said that N-acetyl glyphosate is stable in water or acid for a year. The vendor said they did NMR purity analysis which is 98%.

So, before I get the new shipment, I did fraction collection for pure N-acetyl glyphosate. In water or acid, it is stable in the first hour. Then, I spiked it in carrot for extraction test. It is still fine. I called my organic chemist friend. He told me that it is light sensitive. So, I keep the vials in autosampler for overnight. If it is still pure, I can go ahead for the validations.

TBuAA is also light sensitive. We have the mobile phase in amber bottle.

Best regards,

Eugene

From: Chang, Eugene
To: Sack, Chris A
Subject: It is stable
Date: Wednesday, January 11, 2017 12:38:44 PM

Hi, Chris,

Good news: N-acetyl glyphosate is fine for overnight.

So, I can run a 4-compound together batch today!

I put it in amber volumetric flask anyway. For sample vial and extraction steps, it seems to be fine.

Thanks,

Eugene

From: Chang, Eugene
To: Sack, Chris A
Subject: 10 ng/g of the 4 compounds in carrot
Date: Thursday, January 12, 2017 9:49:55 AM
Attachments: Carrot 10 ng_g 1.pdf

Hi, Chris,

I received 25 mg N-acetyl glyphosate from TRC. This time, I verified their purity first. The results from my fraction collected standard spiked in carrot showed no degradation. I did single compound on Tuesday. This is the result for mixture.

I'll run a full batch tonight.

If Moh asks about the progress, it's the status now. I am still waiting for avocado and carrots. But for time being, I use my clean organic products. So, I may get 2X carrots, 2X avocado and 2X corns.

Thanks,

Eugene

From: Chang, Eugene
To: Thompson, Richard L.
Cc: Sack, Chris A
Subject: RE: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0
Date: Thursday, January 12, 2017 10:02:20 AM

Hi, Richard,

I use glyphosate alone, a single point, to measure N-acetyl glyphosate solution. Then, I did fraction collection to get pure N-acetyl glyphosate. With this pure solution, I expected to see the degradation in water and acid. However, it did not happen. Even after I spiked it in carrot for a real extraction, it was still a single peak.

So, I called the vendor. They told me the 98% pure on their COA is from NMR data – it is reliable. The possibility of degradation is light. The compound might be light sensitive. It explained the breakdown in my stock solution. It was sit there over the holidays under florescence light tube. Now, I got the new ordered 25 mg standard. I put it in amber volumetric flask.

It is clarified. It is stable but light sensitive.

Thanks,

Eugene

From: Chang, Eugene
Sent: Tuesday, January 10, 2017 10:47 AM
To: Sack, Chris A
Cc: Thompson, Richard L.
Subject: RE: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0

Hi, Chris,

Yes, I am using this column.

You can check with Richard. He may still use the 2.1mm column came with API4000. If we try to use that one, everybody has it. It is Luna 5 µ C8, not Luna 5 µ C8(2). But, it is fine. For this method, I did not see difference.

BTW, I tested N-acetyl glyphosate in water and in acid. It hydrolyzes fast to get equilibrium. In water, it's about 1/8 remaining as N-acetyl glyphosate. In acid, it's 1/10 or 1/20 left, depends on pH. I need to order small amount, and dissolve it in methanol to see if it is stable.

Thanks,

Eugene

From: Sack, Chris A
Sent: Tuesday, January 10, 2017 7:10 AM
To: Chang, Eugene
Subject: RE: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0

Hi Eugene,

Some labs are hesitant to buy this column because they want to make sure it is the column we will use for the collaboration. I just want to verify with you that the Luna 5 µ C8(2) 150X4.6mm, part no. 00F-4249-E0 is final?

Thanks,
Chris

Ph: 240-402-2464

From: Chang, Eugene
Sent: Thursday, December 22, 2016 12:10 PM
To: Sack, Chris A
Subject: RE: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0

YES.

Happy Holidays!

Eugene

From: Sack, Chris A
Sent: Thursday, December 22, 2016 9:23 AM
To: Chang, Eugene
Subject: RE: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0

Hi Eugene,

Just checking back with you to see if the preferred column is still : Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0? Is this final?

Thanks,

Chris

Ph: 240-402-2464

From: Chang, Eugene
Sent: Friday, December 16, 2016 11:01 AM
To: Sack, Chris A
Subject: RE: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0

I have 3 Luna columns:

1. Luna C8 narrow – from 4000 QTrap demo column
2. Luna C8 wide – new ordered, came on Wed.
3. Luna C8 (2) wide – from our drug group, borrowed, almost new

The last week tests showed that 1 and 3 worked, I predict 2 should be better.
After 2 came on Wed, the comparison showed that 3 is better than 2.

Part Number: 3 - Luna C8 (2) wide: 00F4249-E0 – this is the primary choice now.
2 – Luma C8 wide: 00F4040-E0

Thanks!

Eugene

From: Sack, Chris A
Sent: Friday, December 16, 2016 8:37 AM
To: Chang, Eugene
Subject: RE: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0

Hi Eugene,

Just to be clear the pic you sent yesterday lists a different part number: 00F-4040-E0 – see attached pic. Which is correct?

Thanks,

Chris

Ph: 240-402-2464

From: Chang, Eugene
Sent: Thursday, December 15, 2016 5:37 PM
To: Sack, Chris A
Subject: Clarify the part number of column: Luna 5 µ C8(2) 150X4.6mm, 00F-4249-E0

No contents

From: Chang, Eugene
To: Thompson, Richard L.
Cc: Sack, Chris A
Subject: FW: 10 ng/g of the 4 compounds in carrot
Date: Thursday, January 12, 2017 5:12:16 PM

Hi, Richard,

I am sorry that I go back and forth. My re-injection of fraction collected compound is stable. It is for sure. However, the purity from TRC shipment has problem. What I saw in the morning looks good, but now I realized that the glyphosate peak shifted out from the window. The injection now shows 9:1 again, that means 10% purity. I asked TRC QC manager show me the data of their COA. He will give me response tomorrow morning.

I am sure the compound is stable. The standard they sent to me was way lower than 98% pure. If you have it, you may estimate the purity of the single standard, too. If some of us have the standard from SCBT, it might be with higher purity. My fraction collection can cover low level spike now, but for the high levels, I have to collect many times – a whole day or two days.

Thanks,

Eugene

From: Chang, Eugene
Sent: Thursday, January 12, 2017 7:50 AM
To: Sack, Chris A
Subject: 10 ng/g of the 4 compounds in carrot

Hi, Chris,

I received 25 mg N-acetyl glyphosate from TRC. This time, I verified their purity first. The results from my fraction collected standard spiked in carrot showed no degradation. I did single compound on Tuesday. This is the result for mixture.

I'll run a full batch tonight.

If Moh asks about the progress, it's the status now. I am still waiting for avocado and carrots. But for time being, I use my clean organic products. So, I may get 2X carrots, 2X avocado and 2X corns.

Thanks,

Eugene

From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Noonan, Gregory
Subject:	Glyphosate method update
Start:	Friday, January 13, 2017 12:00:00 PM
End:	Friday, January 13, 2017 1:00:00 PM
Location:	Telecon

Good Morning,

Let's have a quick meeting to update our progress with the glyphosate method implementation. If you can't make it send me a brief update via email.

Talk soon,

Chris

Chris Sack invites you to an online meeting using WebEx.

Meeting ID: (b) (6)
Meeting Password: (b) (6)

To join this meeting
---
1. Go to https://fda.webex.com/fda/j.php?MTID=m071356f56d5e219a8aff810eafa9367f
2. If requested, enter your name and email address.
3. If a password is required, enter the meeting password: (b) (6)
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5. Follow the instructions that appear on your screen.

Teleconference information
---
1. Provide your number when you join the meeting to receive a call back. Alternatively, you can call one of the following numbers:
Local: (b) (6)
toll free: (b) (6)
2. Follow the instructions that you hear on the phone.
Your Cisco Unified MeetingPlace meeting ID: (b) (6)

FDARichMedia@fda.hhs.gov

Technical support:
Contact FDA Rich Media at 301-796-3333.

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From:	Chang, Eugene
To:	Sack, Chris A; Chamkasem, Narong; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Noonan, Gregory
Subject:	RE: Glyphosate method update - COA of N-acetyl glyphosate and predicted NMR
Date:	Friday, January 13, 2017 10:20:23 AM

I called the vendor to complain the low purity and request for their NMR spectra. They are doing experiment now.

My LCMS showed N-acetyl glyphosate with ~10% pure. ~ 90% is glyphosate (estimate). I'll report the details to you.

Best regards,

Eugene

-----Original Appointment-----
From: Sack, Chris A
Sent: Friday, January 13, 2017 5:24 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Noonan, Gregory
Subject: Glyphosate method update
When: Friday, January 13, 2017 12:00 PM-1:00 PM (UTC-06:00) Central America.
Where: Telecon

Good Morning,

Let's have a quick meeting to update our progress with the glyphosate method implementation. If you can't make it send me a brief update via email.

Talk soon,

Chris

Chris Sack invites you to an online meeting using WebEx.

Meeting ID: (b) (6)

Meeting Password: (b) (6)

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---

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2. If requested, enter your name and email address.

3. If a password is required, enter the meeting password: (b) (6)

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Contact FDA Rich Media at 301-796-3333.

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From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene
Subject:	N-acetyl-glyhphosate
Date:	Friday, January 13, 2017 10:24:00 AM
Attachments:	Glyphosate_RAR_07_Volume_3CA-CP_B-5_2013-12-18_san.pdf Glyphosate_RAR_09_Volume_3CA-CP_B-7_2013-12-18.pdf

Hi Eugene and Narong,

I asked Monsanto about the stability of N-acetyl-glyphosate and they sent me the attached documents. I looked briefly through of the 07 document and I don't see any indication that N-acetyl-glyphosate is unstable.

Chris

From:	Chamkasem, Narong
To:	Sack, Chris A; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Noonan, Gregory
Subject:	RE: Glyphosate method update
Date:	Friday, January 13, 2017 11:28:31 AM

Interesting method using phosphoric acid as the ion-pair reagent on a reversed phase column. No issue with N-acetyl-glyphosate stability has been mentioned.

https://www.google.com/patents/EP2124570A1?cl=en

-----Original Appointment-----
From: Sack, Chris A
Sent: Friday, January 13, 2017 8:24 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Noonan, Gregory
Subject: Glyphosate method update
When: Friday, January 13, 2017 12:00 PM-1:00 PM (UTC-06:00) Central America.
Where: Telecon

Good Morning,

Let's have a quick meeting to update our progress with the glyphosate method implementation. If you can't make it send me a brief update via email.

Talk soon,

Chris

Chris Sack invites you to an online meeting using WebEx.

Meeting ID: (b) (6)

Meeting Password: (b) (6)

To join this meeting

---

1. Go to https://fda.webex.com/fda/j.php?MTID=m071356f56d5e219a8aff810eafa9367f

2. If requested, enter your name and email address.

3. If a password is required, enter the meeting password: (b) (6)

4. Click "Join".

5. Follow the instructions that appear on your screen.

Teleconference information

---

1. Provide your number when you join the meeting to receive a call back. Alternatively, you can call one of the following numbers:

Local: (b) (6)

toll free: (b) (6)

2. Follow the instructions that you hear on the phone.

Your Cisco Unified MeetingPlace meeting ID: (b) (6)

FDARichMedia@fda.hhs.gov

Technical support:

Contact FDA Rich Media at 301-796-3333.

IMPORTANT NOTICE: This WebEx service includes a feature that allows audio and any documents and other materials exchanged or viewed during the session to be recorded. By joining this session, you automatically consent to such recordings. If you do not consent to the recording, discuss your concerns with the meeting host prior to the start of the recording or do not join the session. Please note that any such recordings may be subject to discovery in the event of litigation.

From: Sack, Chris A
To: (b) (6)
Subject: RE: Glyphosate_RAR_09_Volume_3CA-CP_B-7_2013-12-18
Date: Friday, January 13, 2017 12:56:00 PM

Hi Marian,

We would like to include N-acetylglyphosate in our glyphosate method but we seem to be having some problem with the standard. At first we thought maybe the compound was unstable, but the literature doesn't suggest that. Now we think we may have gotten a bad standard. We don't see a lot of vendors for the compound. I was wondering you could recommend (b) (5) or perhaps (b) (4) has the reference material and would be willing to provide some to the FDA for this method development project.

Thanks and have wonderful weekend,

Chris

Ph: 240-402-2464

(b)(4) Consultation

From: Thompson, Richard L.
To: Sack, Chris A
Cc: Cooke, William; Vonderbrink, John; Masse, Claude
Subject: Glyphosate Mobile Phase
Date: Friday, January 13, 2017 1:35:24 PM

Chris,

Glyphosate Mobile Phase A: 4mM tetrabutylammonium hydroxide with the pH adjusted to 2.8 with formic acid.

Using : ACROS Tetrabutylammonium hydroxide titrant 0.4M in water , HPLC Grade Part Number; 420125000 500 mL

Prepare by adding 10.0 mL of the TBA-OH to approximately 990 mL of HPLC water. Using a pH meter adjust the pH to 2.8 with formic acid. It should take about 3 mL of the formic acid. Dilute to 1000 mL with HPLC water.

Glyphosate Mobile Phase B: Acetonitrile HPLC/MS Grade

To the method I added an equilibration step (4 minutes) before each injection to return the column to the initial ion paired state after the previous gradient run.

Let me know if you need any more information.

Richard Thompson
Chemist
US FDA
Arkansas Regional Laboratory
Pesticides Laboratory
Tel 870-543-4054
Richard.thompson@fda.hhs.gov

From: Mercer, Gregory E
To: Thompson, Richard L.; Chang, Eugene; Sack, Chris A
Subject: Luna column
Date: Friday, January 13, 2017 2:05:04 PM

We can't find the column that came with the instrument. We want to place the order today. Which one do you recommend. The 4.6 or 2.0?

From: Chang, Eugene
To: Mercer, Gregory E; Thompson, Richard L.; Sack, Chris A
Subject: RE: Luna column
Date: Friday, January 13, 2017 2:26:00 PM

I prefer 4.6 mm. The reason is that the matrix interference, although we don't monitoring, may overwhelm the narrow column.

From: Mercer, Gregory E
Sent: Friday, January 13, 2017 12:05 PM
To: Thompson, Richard L.; Chang, Eugene; Sack, Chris A
Subject: Luna column

We can't find the column that came with the instrument. We want to place the order today. Which one do you recommend. The 4.6 or 2.0?

From:	Chang, Eugene
To:	Mercer, Gregory E; Thompson, Richard L.; Sack, Chris A
Subject:	RE: Luna column
Date:	Friday, January 13, 2017 2:56:06 PM

However, the 2 mm column gets re-equilibrated faster. I'll follow Richard.

From: Chang, Eugene
Sent: Friday, January 13, 2017 12:26 PM
To: Mercer, Gregory E; Thompson, Richard L.; Sack, Chris A
Subject: RE: Luna column

I prefer 4.6 mm. The reason is that the matrix interference, although we don't monitoring, may overwhelm the narrow column.

From: Mercer, Gregory E
Sent: Friday, January 13, 2017 12:05 PM
To: Thompson, Richard L.; Chang, Eugene; Sack, Chris A
Subject: Luna column

We can't find the column that came with the instrument. We want to place the order today. Which one do you recommend. The 4.6 or 2.0?

From:	Chamkasem, Narong
To:	Sack, Chris A; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Cc:	Noonan, Gregory; Viner, Marianna
Subject:	RE: Draft Minutes for PMC meeting Jan 13, 2017
Date:	Tuesday, January 17, 2017 7:50:36 AM
Attachments:	n-acetyl-glyfosate.pdf

Here's the order form for N-acetyl-glyphosate that I had if you need it.

From: Sack, Chris A
Sent: Monday, January 16, 2017 9:47 AM
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: Draft Minutes for PMC meeting Jan 13, 2017

**********DRAFT**********

Thanks for meeting on short notice. Please review the minutes, but more importantly, I need you to review the attached method to ensure it is correct. Note I highlighted the standard preparation section for special attention.
I will finalize the minutes early this week.
Stay warm,
Chris

****

PesTAG PMC Meeting Minutes

Date: January 13, 2017

(b) (5)

(b) (5)

Happy New Year,

Chris

From: Chamkasem, Narong
To: Sack, Chris A
Subject: FW: N-acetyl-glyhphosate
Date: Tuesday, January 17, 2017 7:56:37 AM

Please share with the group.

Von: Chamkasem, Narong [mailto:Narong.Chamkasem@fda.hhs.gov]
Gesendet: Freitag, 13. Januar 2017 17:46
An: Eichhorn, Eric (CVUA-S)
Betreff: FW: N-acetyl-glyhphosate

Hi Eric:

One of our lab is working on developing method for N-acetyl-glyphosate and he experience stability issue of N-acetyl-glyphosate and he claimed that it will degrade to glyphosate, particularly in acid media. Note that QuPPe use methanol + 1 % formic acid. Any comments?

Regards,

Narong Chamkasem

From: Sack, Chris A
Sent: Friday, January 13, 2017 11:24 AM
To: Chamkasem, Narong; Chang, Eugene
Subject: N-acetyl-glyhphosate

Hi Eugene and Narong,

I asked (b) (4) about the stability of N-acetyl-glyphosate and they sent me the attached documents. I looked briefly through of the 07 document and I don't see any indication that N-acetyl-glyphosate is unstable.

Chris

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:	Status update
Date:	Monday, January 23, 2017 11:48:00 AM

Hi Everyone,

Barring a few tweaks we might find necessary during implementation the glyphosate method is about finalized. At this time all labs are expected to have ordered the requisite supplies and reagents and implemented the LC-MS/MS method on a 5500 or 6500. All labs need to evaluate the linearity, range and precision on their instrument. I recommend you start by determining if your instrument has the necessary sensitivity for the analysis; i.e. to achieve an LOQ of 10 ng/g for the method glyphosate needs to be quantifiable at 2 ng/ml (10 ng/g * 0.2 g/ml). [Note: Only Eugene needs to determine the MDL and associated LOQ based upon the multiple iterations of the extraction procedure as per 40 CFR 136]. To demonstrate instrument sensitivity I propose injecting a low level sensitivity curve at 1, 2, 5 and 10 ng/ml. Determine the LOQ for each level as per ORA-LAB.10; i.e. we just need to know the concentration at 10 x of the S/N for the quant ion assuming the S/N of qualifier ion = 3.

For the high end of the range I would recommend you include standards at concentrations exceeding the range we have agreed to collaborate (500 ng/g = 100 ng/ml solution concentration). Narong's standard curve went up to 250 ng/ml. For precision, I would recommend multiple injections at our calibration level which we have not discussed – Narong and Eugene do you have a recommendation?

If you have different ideas about demonstrating instrumental determination suitability let me know. Please send me a status update. I will plan a phone call later this week at our normal time of 11 AM EST. When you send me your status let me know if you prefer Wed or Thurs.

Thanks

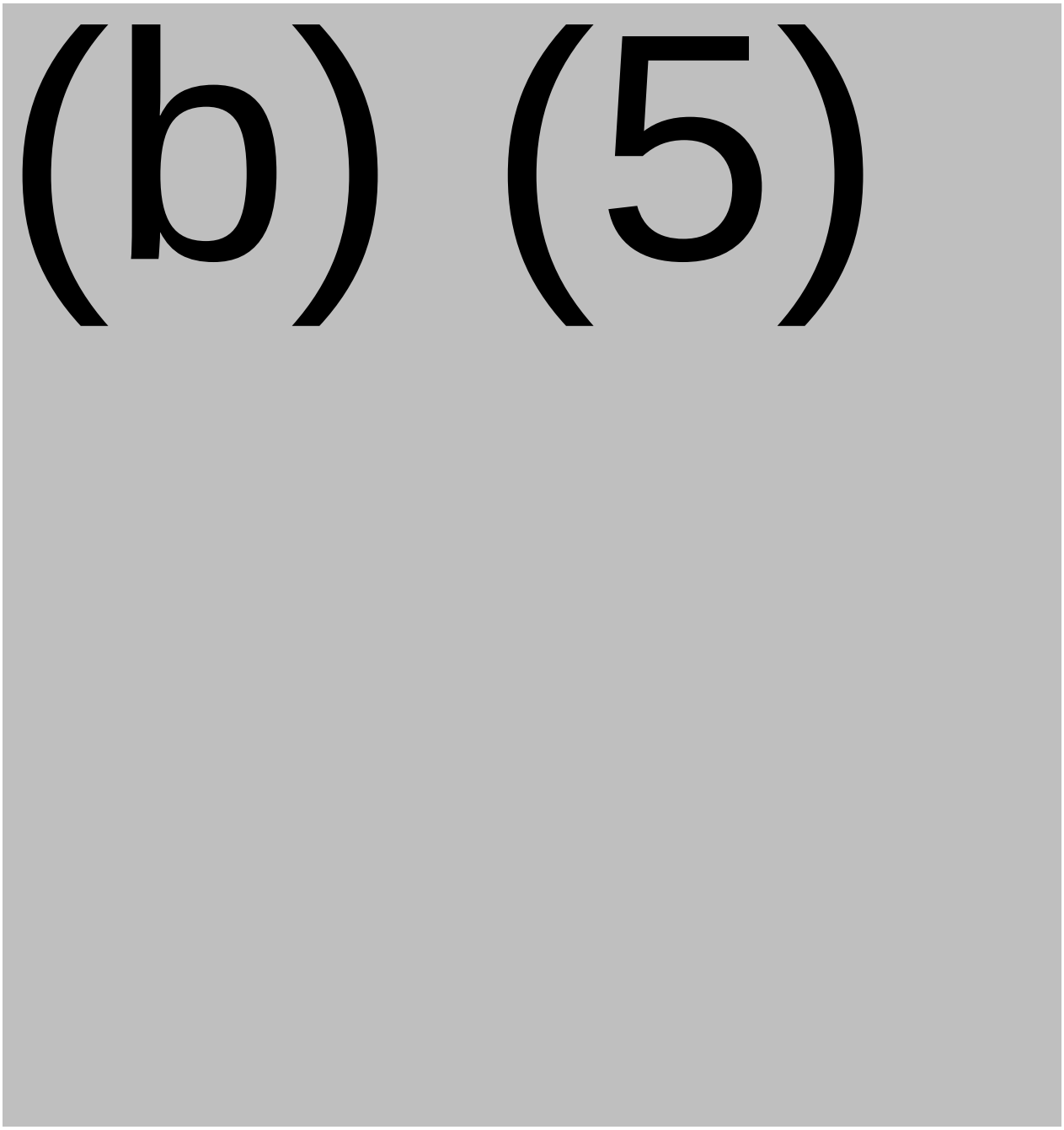
Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Monday, January 23, 2017 10:31 AM
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

PesTAG PMC Meeting Minutes

(b) (5)



Happy New Year,

Chris

From:	Islam, Mohammed R
To:	Sack, Chris A; Chamkasem, Narong; Chang, Eugene; Cooke, William; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Subject:	RE: Glyphosate LOQ
Date:	Tuesday, January 24, 2017 11:26:05 AM

Richard's data looks very convincing using the 2.0 x 150 mm column, at this point all labs please check your instrument sensitivity and report back to us.

I hope we do not need to change any more column, I already heard there is a back order for 4.6x 150 mm column.

Thanks.
Moh

From: Sack, Chris A
Sent: Tuesday, January 24, 2017 11:52 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Subject: FW: Glyphosate LOQ

Wow Richard! Results are amazing. If this sensitivity is duplicated around the country we might consider (b) (5)

Chris

Ph: 240-402-2464

From: Thompson, Richard L.
Sent: Tuesday, January 24, 2017 10:30 AM
To: Sack, Chris A
Subject: Glyphosate LOQ

Chris,

I have attached PDF,s of the S/N chromatograms for; 1.0 ng/mL; 2.0 ng/mL; 5.0 ng/mL, and 10.0 ng/mL of Glyphosate.

The S/N and LoQ's are:

		S/N	LoQ
1.0ng/mL Std	Glyphosate 1	51.7	0.19 ng/mL
	Glyphosate 2	10.7	0.30 ng/mL
2.0 ng/mL Std	Glyphosate 1	50.7	0.39 ng/mL
	Glyphosate 2	121.9	0.47 ng/mL
5.0 ng/mL Std	Glyphosate 1	97.8	0.51 ng/mL
	Glyphosate 2	20.8	0.75 ng/mL

10.0 ng/mL Std	Glyphosate 1	157.7	0.63 ng/mL
	Glyphosate 2	115.5	0.26 ng/mL

I hope this is what you are wanting to see.

This was run on the 2.0 x 150 mm column. I have not put the 4.6x 150 mm column in the instrument yet.

Regards,

Richard Thompson
Chemist
US FDA
Arkansas Regional Laboratory
Pesticides Laboratory
Tel 870-543-4054
Richard.thompson@fda.hhs.gov

From: Vonderbrink, John
To: Sack, Chris A
Subject: RE: Carrots
Date: Tuesday, January 24, 2017 1:28:39 PM
Attachments: image001.png

Hi Chris,

No worries at all. We're happy for you. It's been stressful around here lately and I've been given other assignments by our LD which must be completed before I can get into glyphosate. So to make a long story short, I have not run anything yet. I have the standards being made and will inject them tomorrow. If all goes well then I will inject the low standards to check LOQ. I have the carrots thawing and either Mark Ross or Stacy Hetz will get the samples sent out tomorrow as well.

That brings me back to our conversation last Wednesday about the 4465 collaboration report. Do you still have a copy of the report or summary tables? If so can you send a copy to me. Thanks.

I'll keep you posted.

John

John Vonderbrink
Chemist—Pesticides

Office of Regulatory Affairs
Kansas City Laboratory
U.S. Food and Drug Administration
Tel: 913-752-2703
john.vonderbrink@fda.hhs.gov

From: Sack, Chris A
Sent: Tuesday, January 24, 2017 11:01 AM
To: Vonderbrink, John
Subject: Carrots

Hi John,

Hope all is well. It was good to see you and Jeannie. I hope I didn't seem to smug about current job situation. I can't tell you how many days I would get home from lab frustrated and/or furious – it would take me about 10-15 miles into a bike ride to bring BP down. Jeannie was right, it is much easier now that I don't report to the lab.

Do you have any glyphosate results yet? Can you send me a status update? Also, did you ship the carrots?

Thanks,

Chris

From: Sack, Chris A
To: Robin, Lauren P
Subject: RE: followup
Date: Tuesday, January 24, 2017 1:36:00 PM
Attachments: image001.png

Hi Lauren,

I requested an update from all labs yesterday. Two ORA labs are ready to run the method. Two more ORA labs should be ready by end of week. One ORA lab has been diverted, one ORA lab is setting up. CFSAN has ordered supplies. All labs will have the collab samples by end of this week. I will have a meeting with them all this Thursday AM.

Chris

Ph: 240-402-2464

From: Robin, Lauren P
Sent: Tuesday, January 24, 2017 10:41 AM
To: Sack, Chris A
Subject: followup

Hi Chris

Did you get a chance to put together a summary of the glyphosate methodology status?

Lauren

Lauren Posnick Robin, Sc.D.
Chief, Plant Products Branch
DPPB/OFS/CFSAN
U.S. Food and Drug Administration
HFS-317
5001 Campus Drive
College Park, MD 20740
240-402-1639
lauren.robin@fda.hhs.gov

From: Sack, Chris A
To: Robin, Lauren P
Subject: RE: followup
Date: Tuesday, January 24, 2017 1:53:00 PM
Attachments: image001.png

The two labs that have done the sensitivity testing on the instruments are reporting sub 10 ppb level equivalence. By the end of the week I hope to know if sensitivity can be achieved in two more labs. The validating lab (LA) tells me they should have a validation report by end of week. I assume the results are satisfactory, otherwise they would have notified me. Hopefully, next week two-three more labs will have some recovery data. If I can get three labs demonstrating good sensitivity and recovery data by end of next week I hope to get the collab started the week after (Feb 6). Assuming all that goes well, it will take a few weeks to work up the data. So we are looking at the end of Feb. That would be my best guesstimate right now.

Chris

Ph: 240-402-2464

From: Robin, Lauren P
Sent: Tuesday, January 24, 2017 1:38 PM
To: Sack, Chris A
Subject: RE: followup

What LOQ?

What is the timing on when the assignment can restart (earliest)?

From: Sack, Chris A
Sent: Tuesday, January 24, 2017 2:36 PM
To: Robin, Lauren P
Subject: RE: followup

Hi Lauren,

I requested an update from all labs yesterday. Two ORA labs are ready to run the method. Two more ORA labs should be ready by end of week. One ORA lab has been diverted, one ORA lab is setting up. CFSAN has ordered supplies. All labs will have the collab samples by end of this week. I will have a meeting with them all this Thursday AM.

Chris

Ph: 240-402-2464

From: Robin, Lauren P
Sent: Tuesday, January 24, 2017 10:41 AM
To: Sack, Chris A
Subject: followup

Hi Chris

Did you get a chance to put together a summary of the glyphosate methodology status?

Lauren

Lauren Posnick Robin, Sc.D.
Chief, Plant Products Branch
DPPB/OFS/CFSAN
U.S. Food and Drug Administration
HFS-317
5001 Campus Drive
College Park, MD 20740
240-402-1639
lauren.robin@fda.hhs.gov

From: Chamkasem, Narong
To: Sack, Chris A; Chang, Eugene
Subject: RE: Glyphosate cleanup
Date: Wednesday, January 25, 2017 8:08:17 AM
Attachments: IMG_2925.JPG
IMG_2928.JPG
IMG_2931.JPG
IMG_2935.JPG
IMG_2921.JPG
IMG_2922.JPG

Chris

I did tried it with flour because the lab in Australia had issue of filtering it on the Oasis HLB. I shook the flour with solvent, centrifuged really well, and passed the extract thru the SPE. One of the picture shows the comparison of the two where UCT (left) gave a cloudy extract and Oasis (right) is clear. I did not check the recovery of the UCT because I did not have time. With UCT, you will need a syringe to suck the sample and pass it thru, while the Oasis you pipette the extract into the SPE and use a pipette bulb or air (from the Tygon tubing) push the extract thru, your choice.

Narong

From: Sack, Chris A
Sent: Wednesday, January 25, 2017 8:12 AM
To: Chamkasem, Narong; Chang, Eugene
Subject: Glyphosate cleanup

Eugene and Narong,

FYI. Our UCT sales rep shared this with me yesterday.

Chris

Ph: 240-402-2464

(b)(4) Consultation

(b)(4) Consultation

From:	Chang, Eugene
To:	Sack, Chris A
Cc:	Thompson, Richard L.
Subject:	Validation data, partial
Date:	Wednesday, January 25, 2017 5:07:45 PM
Attachments:	20170124corn.pdf 20170124 corn linearity.pdf avocado.pdf avocado interference and solution.pdf 20170124 Corn.xlsx Carrot_glyphosate only.xlsx

---

Hi, Chris,

Attached is a set of my “validation”. I have to inject the extraction vials again because I repaired the LC pump B. The RT is changed.

Corn has 3 files: chromatograms, calibration lines and data-calculations. Carrot has 1 file, nothing special, all data are as good as corn.

Avocado has 2 files. In the middle of the run, I realized the pump did not get enough flow for acetonitrile. The confirmation ion 180-85 has interference from matrix. However, 180-95 is good, so I need to add it back. Its height is the same as 180-85.

I have isotopic IS for glyphosate and glufosinate. For AMPA, I can use either one of them as IS, or not use IS. If I don’t use IS, the recovery of AMPA is about 50%. With glyphosate IS, it is higher. With glufosinate IS, it is also about 50%.

If I use isotopic glyphosate IS for all 3, glufosinate concentration will be driven too high. I called it “mismatched IS calculation” on the spreadsheet.

Overall speaking, the method performance is good.

Best regards,

Eugene

From: Sack, Chris A
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Subject: Some initial validation results from LA
Date: Thursday, January 26, 2017 8:30:00 AM

Hi everyone,

Just wanted you to see some initial validation results I received from LA yesterday.

Eugene provided spiked carrots, corn and avocado each, 7 replicates @ 20 ng/g, and 3 replicates each at 50, 200, and 500 ng/g. In the table below MDLs and LOQs were calculated based upon the 20 ng/g spikes. Recovery, RSD and Linearity were calculated based upon all recoveries. Note AMPA stats based upon external standard calibration, all others based upon isotopic internal standard calibration.

	Carrot	**Corn**		
	Glyphosate	**Glyphosate**	**Glufosinate**	**AMPA**
MDL (ng/g)	3.5	2.1	2.4	2.0
LOQ (ng/g)	11.4	7.0	8.0	6.5
Recovery	105	101	101	43
RSD	4.1	4.5	3.2	18.9
Linearity	0.9996	0.9995	0.9997	0.9327

Excellent work Eugene and LA crew! For our meeting today I need everyone to provide an update on the progress made to implement the glyphosate method in their labs.

Talk soon,

Chris

From: Cooke, William
To: Sack, Chris A
Cc: Mercer, Gregory E
Subject: RE: Some initial validation results from LA
Date: Thursday, January 26, 2017 10:01:55 AM
Attachments: image002.png

Morning Chris,

We've been using the Luna C8(2) 2.0mm x 150mm 5um column (P/N 00F-4249-B0) with the guard column. I'm using the same gradient but (copied below) but matching Richard's column oven (35C) and flow rate (300 uL/min).

Retention times have been stable in extraction solution and Narong's corn matrix (at 10 ng/g in matrix matched standard). The glyphosate interference in corn is better resolved than the phenyl column. One thing is the peaks are very wide, glyphosate is at 30 seconds at the base.

My RTs are
AMPA 1.3
Glufosinate 2.6
Glyphosate 4.0
N-acetyl glyphosate 5.2

I'm working on reducing peak width without changing the MP A concentration (checking for voids, reducing dead volume, oven temp, flow rate). Injecting 1 uL of standard vs 10 uL hasn't affected peak width. Hopefully can increase S/N with tighter peaks and further separate the corn interference.

We had an EPA NPSR standard of N-acetyl glyphosate along with the TRC source. I prepped them both in LC-MS water and diluted them to the same concentration in water and acidified extraction solution and injected each individually. Peak areas were consistent and didn't show any glyphosate, AMPA, or glufosinate in the chromatogram.

Gradient
A: 4mM TBA-OH adjusted to pH 2.8 in water
B: Neat MeCN

Time	MPB
0.00	5
1.00	5
5.00	95
6.50	95
6.60	5
10.00	5

William "Bill" Cooke
Chemist

Office of Regulatory Affairs
Pacific Regional Laboratory Northwest
U.S. Food and Drug Administration

From:	Chang, Eugene
To:	Vonderbrink, John
Cc:	Sack, Chris A; Chamkasem, Narong; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.
Subject:	pH effect
Date:	Thursday, January 26, 2017 1:19:26 PM
Attachments:	pH and peaks.pdf

Hi, John,

I did a test earlier this month for different pH with 2.0 mm column, attached.

Now, it can tell me that my current pH in mobile phase A is there at 2.8, or to low/too high. If the glyphosate peak is much lower than that of glufosinate, and the retention time is too close, pH is too low. Otherwise, if the peak heights are equal, and the RT is apart more, pH is too high. The peak width also shows difference.

Best,

Eugene

From: Chang, Eugene
To: Sack, Chris A; Chamkasem, Narong; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Cc: Drake, Connie P.
Subject: RE: STANDARD SOLUTION preparation
Date: Friday, January 27, 2017 12:24:26 AM
Attachments: gly sop section 1C.doc

Hi,

Attached file is the draft of SOP for section C – prepare the standard solutions.

About the mobile phase A, three reagents of TBuAOH or TBuAA have no big difference. 0.4 M TBuAOH has small volume, when I use 10 mM, 1 bottle can only get 3 liters of mobile phase A. The 10 gram bottle of TBuAA salt has the same problem. The 1 M TBuAA solution is ease of use: Add 4 mL into 1 L D.I. water, adjust pH to 2.8 with formic acid (about 1.5 to 2 mL depends on the purity, 85% or 98%). A 50 mL bottle can last for 12 liter mobile phase A prep.

The collision energy for Glufosinate, 180-95, should be 18-20 V. It will get the same signal strength as 180-85. My earlier CE was too high.

FYI: After my LC pump B was fixed, I realized that the optimized range of total flow is 0.45 mL/min, and the ramp up acetonitrile percentage is 90% instead of 95%. This setting gets the same back pressure as the previous good runs. I re-injected all three sets of extractions overnight. I'll switch the column back to 2mm C8 tomorrow to do the injection for comparison. I'll keep you posted.

Best regards,

Eugene

From: Sack, Chris A
Sent: Thursday, January 26, 2017 12:48 PM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Cc: Drake, Connie P.
Subject: Minutes for PesTAG PMC meeting January 26, 2017

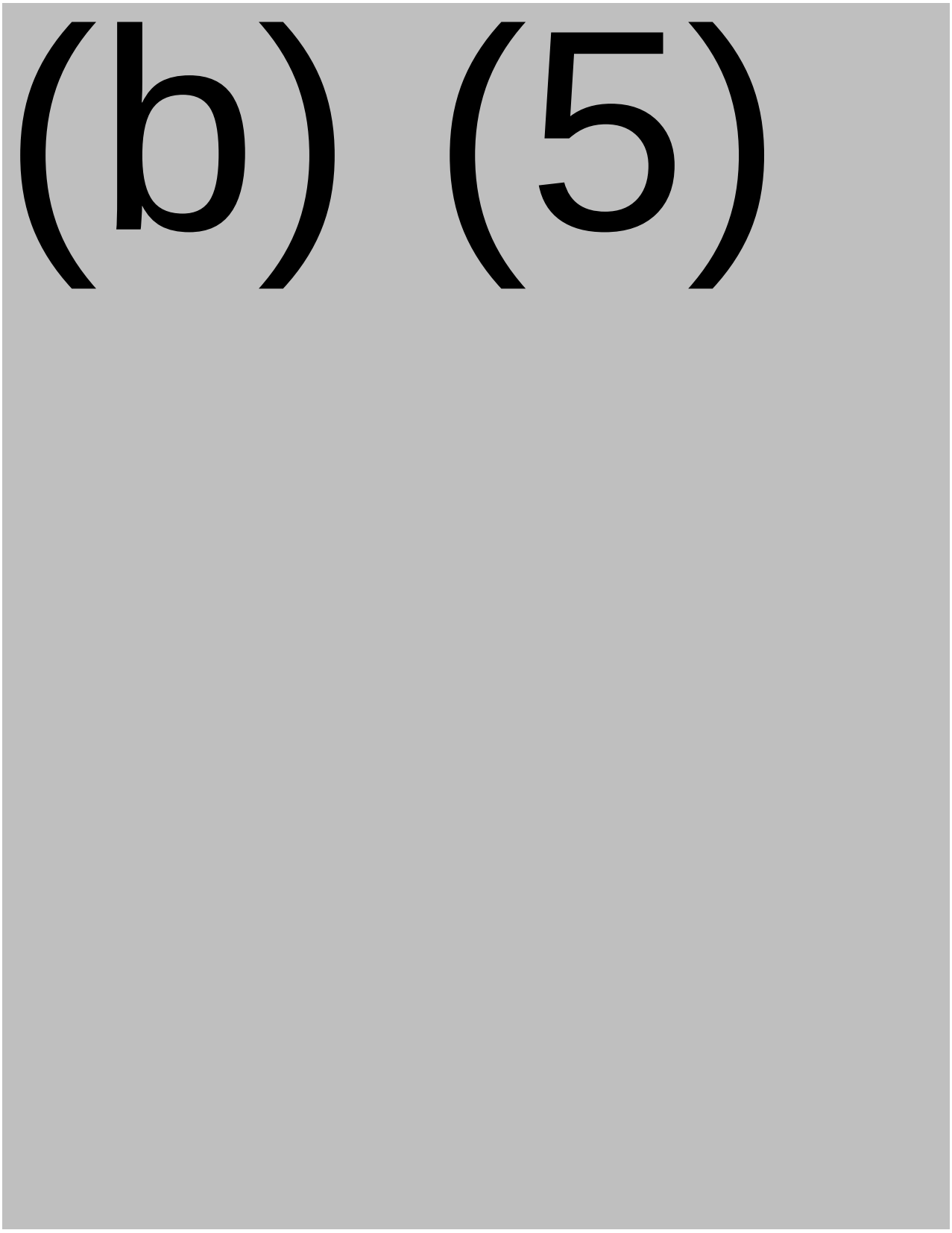
**********DRAFT**********

Please review the minutes and send me correction by first of next week.
Thanks,
Chris

PesTAG PMC Meeting Minutes

Date: January 26, 2017

(b) (5)



Chris

From:	Noonan, Gregory
To:	Sack, Chris A
Cc:	Wong, Jon; Parker, Christine
Subject:	Glyphosate
Date:	Friday, January 27, 2017 7:51:55 AM
Attachments:	Glyphosate Collaboration Details.docx

Chris,
Attached is what I have as the collaboration protocol (I have the method in a separate document). I am sending this to answer some questions Jon had, but I still have a few questions, and Jon, who will be working on the samples, may have a few more.

1. I have received avocado and carrot is on the way, but haven't gotten any corn, should I just contact Narong directly?
2. I don't see a list of the calibration standards you want prepared for the method. Are you leaving the exact number, concentration, and range to each lab or did I miss it?
3. Can you add Jon to the list of collaborators. It was unclear who from CFSAN would be participating, but with some recent changes, Jon has agreed to coordinate the lab work.

I have also included Christine Parker on this email. She is the acting Branch Chief of the Bioanalytical Methods Branch. Basically, she is busy trying to clean up the mess Greg Mercer left behind☺.
Thanks,
Greg

Gregory O. Noonan, PhD
Director, Division of Bioanalytical Chemistry
Food and Drug Administration
5001 Campus Drive, HFS 715
College Park, MD 20740

PH: 240-402-2250
FAX: 301-436-2634
Mobile: 240-701-7415
Gregory.Noonan@fda.hhs.gov

From: Sack, Chris A
To: Chamkasem, Narong
Cc: Noonan, Gregory; Wong, Jon
Subject: Collab samples for CFSAN
Date: Friday, January 27, 2017 8:47:00 AM

Hi Narong,

CFSAN is setting up to participate in the glyphosate collaboration. Would you mind shipping them the collab samples, including the corn and soy samples containing incurred residues, you sent everyone else? You can ship them to Greg Noonan at the address below.

Greg Noonan	5001 Campus Drive, College Park, MD 20740	(240) 402-2250

Thanks and have a wonderful weekend,

Chris

From: Vonderbrink, John
To: Sack, Chris A
Subject: RE: Quick update
Date: Wednesday, February 01, 2017 9:24:16 AM
Attachments: image001.png

Hi Chris,

I've spent the past few days getting acquainted with the method. It appears to be running fine. Linearity with the standards 2 ng/ml – 250 ng/ml is >0.99 for all three compounds. I'm now turning to LOQ. I'll keep you posted.

RCJH tonight!

John

John Vonderbrink
Chemist—Pesticides

Office of Regulatory Affairs
Kansas City Laboratory
U.S. Food and Drug Administration
Tel: 913-752-2703
john.vonderbrink@fda.hhs.gov

From: Sack, Chris A
Sent: Tuesday, January 31, 2017 10:02 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John; Wong, Jon; Noonan, Gregory
Subject: Quick update

Hi everyone,

Just wanted to give you a quick update of some data that is coming in.

Eugene's validation data is summarized below. Data looks amazing Eugene!

	Corn	Carrot	Avocado	Avg
	Glyphosate			
MDL (ng/g)	2.1	1.9	1.7	1.9
LOQ (ng/g)	7.0	6.2	5.7	6.3
Recovery	101	104	101	102.2
RSD	4.5	4.4	5.2	4.7
Linearity	0.9995	0.9998	0.9998	0.9997

	Glufosinate			
MDL (ng/g)	2.4	2.3	2.3	2.3
LOQ (ng/g)	8.0	7.5	7.5	7.7
Recovery	101	102	101	101.4
RSD	3.2	3.4	2.8	3.1
Linearity	0.9997	0.9996	0.9999	0.9997

	AMPA			
MDL (ng/g)	2.0	1.1	1.4	1.7
LOQ (ng/g)	6.5	3.7	4.5	5.5
Recovery	43	15.4	19.0	31.2
RSD	18.9	23.9	16.0	17.5
Linearity	0.9327	0.9990	0.9997	0.9662

ARL provided their instrument proficiency data. I summarized in the table below. Great job Richard!

	Glyphosate	**Glufosinate**	**AMPA**
Lq (ng/ml)	0.2	0.3	0.2
Accuracy	100.3	99.8	100.5
Precision	6.3	1.9	11.9
Linearity	0.9970	0.9999	0.9985

Richard injected standards at 1, 2, 5, and 10 ng/ml for the Lq data. The Lq for each level was fairly consistent. The Lq was calculated per the ORA-LAB.10 instructions; i.e. the lowest level that meets the S/N requirements of 10*S/N of the quantitation ion OR 3*S/N of the confirmation ion. Accuracy and precision were calculated from 8 replicates on the injection of a 50 ng/ml standard. And linearity correlation coefficient was calculated from standards injected at 1-200 ng/ml.

From: Sack, Chris A
To: Cromer, Michele
Subject: RE: columns for glyphosate
Date: Wednesday, February 01, 2017 3:09:00 PM
Attachments: Glyphosate method 1-31-17.docx

Hi Michele,

Hope all is well with you and your family. Both of the columns are listed in the attached method.

Have a great day,

Chris

Ph: 240-402-2464

From: Cromer, Michele
Sent: Wednesday, February 01, 2017 2:29 PM
To: Sack, Chris A
Subject: FW: columns for glyphosate

Hi Chris,
Richard Thompson is out of the office until February 6. We wanted to try the column that he is using and I thought John said it was just like the Luna we are using except with a smaller diameter…around 2.1mm.
We have Luna® 5 µm C8(2) 100 Å, LC Column 150 x 4.6 mm, by Phenomenex.
Do you know what he was using? I wanted to put in an order for one.
Thanks,
Michele

From: Cromer, Michele
Sent: Wednesday, February 01, 2017 2:25 PM
To: Thompson, Richard L.
Subject: columns for glyphosate

Hi Richard,
What is the column that you use now for Glyphosate? We have Luna® 5 µm C8(2) 100 Å, LC Column 150 x 4.6 mm, by Phenomenex. Do you use the same column with a smaller diameter? If yes, can you tell me the Part No.? I'm having a hard time finding it on Phenomenex website.
Thanks,
Michele

From:	Sack, Chris A
To:	Mercer, Gregory E
Subject:	Spiking protocol for collab
Date:	Thursday, February 02, 2017 1:24:00 PM
Attachments:	Collab-Glyphosate 2-3-16.xlsx Glyphosate method 1-31-17.docx

---

Hi Greg,

Would you mind reviewing the attached spiking protocol and let me know what you think? It is just a rude draft and I want to run it by you first, then take it to Eugene for his thoughts. Would love to discuss if/when you have a minute. I have also attached the latest version of the method which includes my standard prep for routine analysis.

I am sure you will have questions,

Chris

From: Mercer, Gregory E
To: Sack, Chris A
Subject: RE: Spiking protocol for collab
Date: Friday, February 03, 2017 11:52:30 AM
Attachments: Glyphosate method 1-31-17_GM.docx

I think the planned fortifications are fine and you already ran that by Shaun and Greg Noonan – right? My only thought was to be even more deliberate and prescriptive regarding the preparation of standard solutions. How the spikes are done and even how the matrix std vials are prepared.

I attached a revised version with a couple examples of what I'm talking about…

Good to talk with you this morning. [redacted] (b) (6)

Have a great weekend.

-Greg

From: Mercer, Gregory E
Sent: Friday, February 03, 2017 4:37 AM
To: Sack, Chris A
Subject: RE: Spiking protocol for collab

[redacted] (b) (6) … Do you want to discuss this now? I'm at home…

From: Sack, Chris A
Sent: Thursday, February 02, 2017 11:24 AM
To: Mercer, Gregory E
Subject: Spiking protocol for collab

Hi Greg,

Would you mind reviewing the attached spiking protocol and let me know what you think? It is just a rude draft and I want to run it by you first, then take it to Eugene for his thoughts. Would love to discuss if/when you have a minute. I have also attached the latest version of the method which includes my standard prep for routine analysis.

I am sure you will have questions,

Chris

From: Sack, Chris A
To: Masse, Claude
Subject: FW: Glyphosate Update
Date: Wednesday, February 08, 2017 7:10:00 AM

Hi Claude,

Can you send me your LCMS proficiency results in a xls file?

Thanks,

Chris

Ph: 240-402-2464

From: Sack, Chris A
Sent: Monday, February 06, 2017 10:43 AM
To: Masse, Claude
Cc: Viner, Marianna; Islam, Mohammed R
Subject: RE: Glyphosate Update

Hi Claude,

Chroms look pretty good. When you are finished with the instrument proficiency download the results into a xls file and send it to me.

Thanks,

Chris

Ph: 240-402-2464

From: Masse, Claude
Sent: Monday, February 06, 2017 10:33 AM
To: Sack, Chris A
Cc: Viner, Marianna; Islam, Mohammed R
Subject: RE: Glyphosate Update

Yes, I forgot to attach it.

From: Sack, Chris A
Sent: Monday, February 06, 2017 11:17 AM
To: Masse, Claude
Subject: RE: Glyphosate Update

Hi Claude,

Did you have an attachment?

Chris

Ph: 240-402-2464

From: Cooke, William
Sent: Wednesday, February 08, 2017 9:29 AM
To: Sack, Chris A
Subject: RE: Glyphosate method and collab

Morning Chris,

I misread my calendar last week, the PM from Sciex is this coming Monday and expected to be finished that Tuesday or Wednesday.

Once they've tuned up the instrument we can get started with the collaboration.

William "Bill" Cooke
Chemist

Office of Regulatory Affairs
Pacific Regional Laboratory Northwest
U.S. Food and Drug Administration
Office: (425) 487-5324

William.Cooke@fda.hhs.gov

From: Sack, Chris A
Sent: Wednesday, February 08, 2017 5:26 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: Glyphosate method and collab

Hi everyone,

Re the method and collab I heard back from CFSAN, ARL and LA, and the attached method and collab protocol has been updated. The collab protocol is fairly prescriptive, so let me know your thoughts.

Re the LCMS proficiency, I have data from ARL and PNW only. I have not received any method proficiency data from anyone. We cannot begin the collab until we have acceptable LCMS and method proficiency data from at least 3 labs.

Thanks,

Chris

From:	Sumter, Jeffery
To:	Sack, Chris A
Subject:	RE: Herbicides Assignment
Date:	Wednesday, February 08, 2017 8:30:58 AM

Thanks for the update, sounds like it all working out.

Happy New Year.

From: Sack, Chris A
Sent: Wednesday, February 08, 2017 9:12 AM
To: Sumter, Jeffery
Cc: Islam, Mohammed R
Subject: RE: Herbicides Assignment

Hi Jeffery,

If all goes well I think we can start up the first of March. We are hoping to start the national method collaboration soon. I should have enough data to prepare a partial collaboration report before the end of Feb. We will need to revise the acid herbicide to change the analytical labs where the glyphosate samples are shipped and analyzed. That is an ORA decision, I am working with them to sort that out.

Happy new year,

Chris

Ph: 240-402-2464

From: Sumter, Jeffery
Sent: Wednesday, February 08, 2017 8:02 AM
To: Sack, Chris A
Subject: RE: Herbicides Assignment

Hello Chris,

How are things going with the Herbicides Assignment?

From: Sack, Chris A
Sent: Thursday, September 22, 2016 12:28 PM
To: Sumter, Jeffery; Islam, Mohammed R
Subject: RE: Herbicides Assignment

Hi Jeffery,

It was good to visit with you. What if you change January 2017 to “calendar year 2017”. And add a statement indicating that (b) (5)
(b) (5) It’s just a suggestion but it allows us some wiggle room for completion and approval of the collaboration.

Thanks,

Chris

Ph: 240-402-2464

From: Sumter, Jeffery
Sent: Thursday, September 22, 2016 11:09 AM
To: Sack, Chris A; Islam, Mohammed R
Subject: Herbicides Assignment

Hello,

A decision has been made to hold the Herbicides Assignment in abeyance until January 2017. CFSAN will draft a memo that explains the reason for the abeyance (i.e., updated multi-lab methodology), effective date (i.e., October 1), and a NLT date for resuming the assignment.

Is there any additional information you would like to add? Are you okay with resuming the assignment NLT January 15? (or suggest another date).

Thank you,

Jeffery

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
Subject:	FW: Glyphosate method and collab
Date:	Thursday, February 09, 2017 9:31:00 AM
Attachments:	image004.png AMPA IS filter.PNG Glufosinate IS filter.PNG Glyphosate Filter.PNG Glyphosate IS filter.PNG

FYI. Good work, Bill. Evidently the GD/X and nylon filters retain glyphosate. Is anybody using PVDF filters? I believe most labs use PTFE for pesticides, correct? If that is the case, it looks like the results from the PTFE filters are OK.

Chris

Ph: 240-402-2464

From: Cooke, William
Sent: Wednesday, February 08, 2017 5:43 PM
To: Sack, Chris A
Subject: RE: Glyphosate method and collab

I'll get started on that with the 200 ng/g spikes and have that out by end of week.

I was looking at the method and didn't notice until now – under supplies #13 the filters are listed as nylon with 2 micron and GD/X. When I was trying to track down my lower response I compared the different filter types. I prepped a few mLs of 25ng/mL standard solution and ran 300 uL through each filter. The GD/X filters have glass microfibers, which retain the glyphosate. The calculated concentration remains the same but both the glyphosate and IS decrease proportionally. Nylon had the same behavior. PVDF without the GD/X portion did not absorb the glyphosate.

We might want to avoid nylon and GMF filters.

Thanks,

William "Bill" Cooke
Chemist

Office of Regulatory Affairs
Pacific Regional Laboratory Northwest
U.S. Food and Drug Administration
Office: (425) 487-5324

William.Cooke@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Sack, Chris A
Sent: Wednesday, February 08, 2017 7:34 AM
To: Cooke, William
Subject: RE: Glyphosate method and collab

Hi Bill,

I need to see some spike recovery data from PNW before your start the collab. One spike from each collab matrix would be fine. You can use the solvent standard calibration at the same level as the spike. Just need evidence that you can run the method and get satisfactory results.

Thanks,

Chris

Ph: 240-402-2464

From: Cooke, William
Sent: Wednesday, February 08, 2017 9:29 AM
To: Sack, Chris A
Subject: RE: Glyphosate method and collab

Morning Chris,

I misread my calendar last week, the PM from Sciex is this coming Monday and expected to be finished that Tuesday or Wednesday.

Once they've tuned up the instrument we can get started with the collaboration.

William "Bill" Cooke
Chemist

Office of Regulatory Affairs
Pacific Regional Laboratory Northwest
U.S. Food and Drug Administration
Office: (425) 487-5324

William.Cooke@fda.hhs.gov

From: Sack, Chris A
Sent: Wednesday, February 08, 2017 5:26 AM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: Glyphosate method and collab

Hi everyone,

Re the method and collab I heard back from CFSAN, ARL and LA, and the attached method and collab protocol has been updated. The collab protocol is fairly prescriptive, so let me know your thoughts.

Re the LCMS proficiency, I have data from ARL and PNW only. I have not received any method proficiency data from anyone. We cannot begin the collab until we have acceptable LCMS and method proficiency data from at least 3 labs.

From:	Chamkasem, Narong
To:	Sack, Chris A; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject:	RE: Glyphosate method and collab
Date:	Thursday, February 09, 2017 3:00:06 PM
Attachments:	image002.png image003.png

My filter is 25 mm size and it will take 2 mL to pass in order to get 0.5 mL out of it. How big is Bill's filter? Bill used two filters piggy bag together (PVDF/GD/X) as well.

Narong

From: Sack, Chris A
Sent: Thursday, February 09, 2017 3:56 PM
To: Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: RE: Glyphosate method and collab

Narong filtered 2 ml at 100ng/ml vs Bill filtered 300ul at 25 ng/ml. Bill, I assume you prepared the standard in the extraction solvent? Maybe the affect can only be seen at lower levels? That's fairly common.

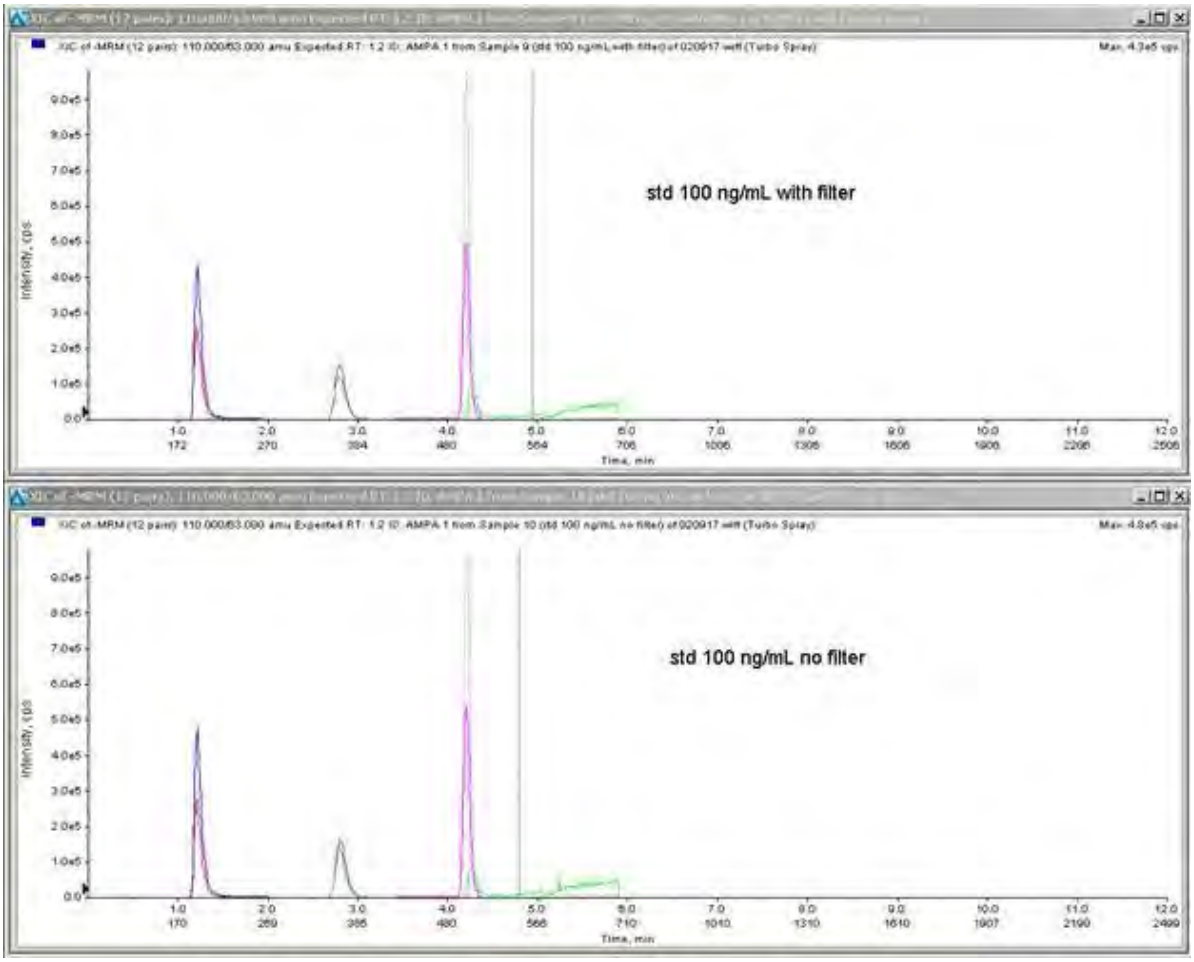
Chris

Ph: 240-402-2464

From: Chamkasem, Narong
Sent: Thursday, February 09, 2017 2:38 PM
To: Sack, Chris A; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: RE: Glyphosate method and collab

I did an experiment by making 100 ng/mL standard mix in the extracting solvent and pass 2 mL thru the filter (GD/X) and inject against the non-filter.

	glyphosate	AMPA	glufosinate
std 100 ng/mL with filter	4700000	2600000	1310000
std 100 ng/mL no filter	5060000	2870000	1350000



The only time that I use filter is for egg method and recovery is good (below)

Table 5. Recovery (%) and RSD (%) data obtained in the validation experiments (n = 7).

Analyte	Fortification level (µg/g)	Recovery (%)	RSD (%)
AMPA	0.05	97.4	6.1
	0.1	92.7	4.9
	0.5	93.7	2.9
	1	94.1	2.5
Glufosinate	0.05	88.2	1.5
	0.1	88.9	2.6
	0.5	89.5	1.9
	1	91.9	3.4
Glyphosate	0.05	92.3	4.1
	0.1	89.5	2.5
	0.5	90.9	2.7
	1	89.0	2.8

From: Chamkasem, Narong
Sent: Thursday, February 09, 2017 2:38 PM
To: Sack, Chris A; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Subject: RE: Glyphosate method and collab

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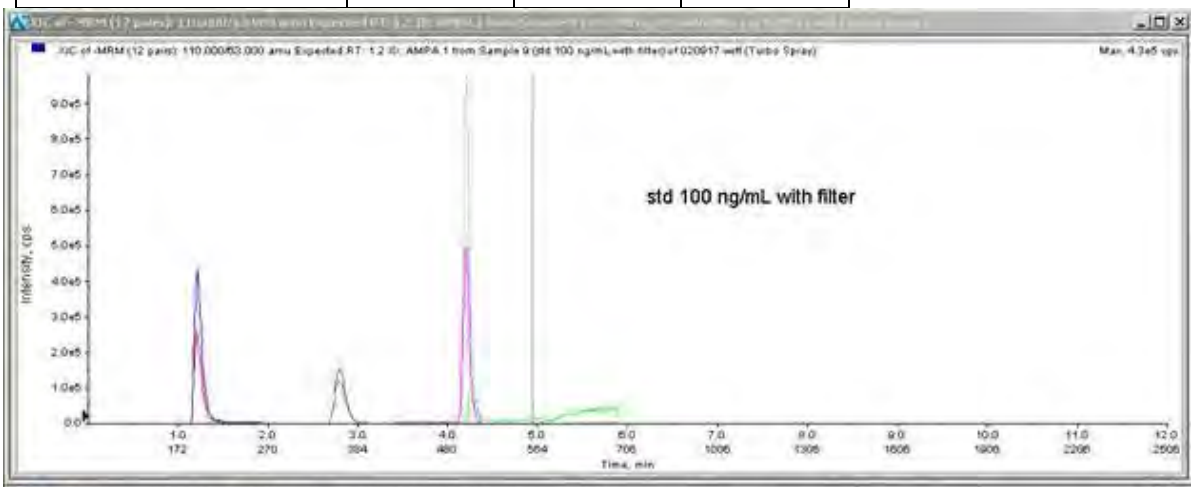
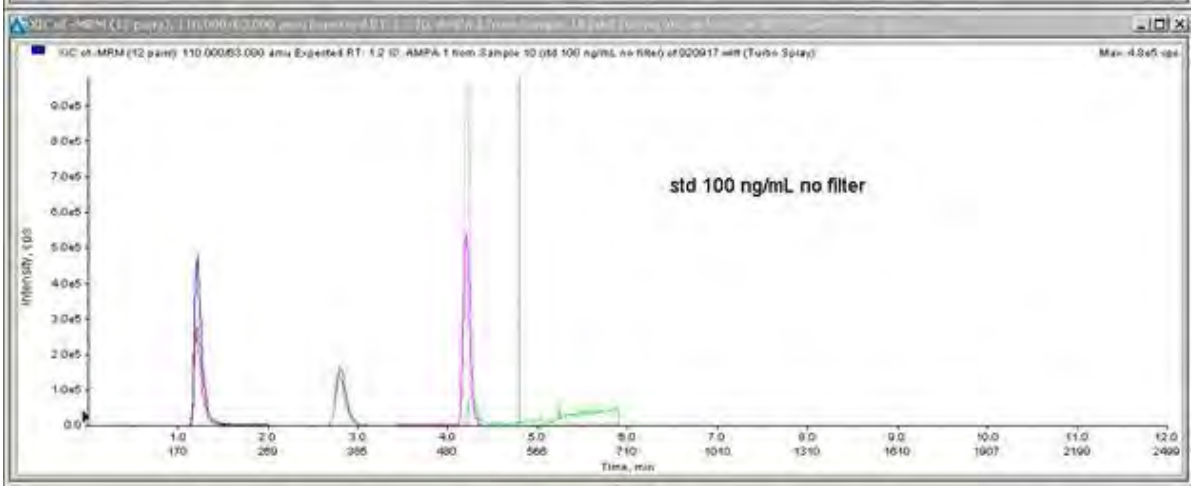




The only time that I use filter is for egg method and recovery is good (below)

From: Cooke, William
To: Sack, Chris A
Cc: Mercer, Gregory E; Mabry-Smith, Ronald C
Subject: Initial spike recovery data
Date: Friday, February 10, 2017 11:15:06 AM
Attachments: 20170209 Recovery.docx
20170209 PRL-NW Small Spike Recovery.xlsx
image002.png

Morning Chris,

Here's the data for a small extraction set on all 3 matrices at 200 ng/g.

The first tab is as you specified in the method with AMPA and N-acetyl compared against native standards and Glufosinate and Glyposate against their respective isotopes. The second tab is with all 4 using isotopes.

AMPA	AMPA IS
Glufosinate	Glufosinate IS
Glyphosate	Glyphosate IS
N-acetyl glyphosate	Glyphosate IS

The extraction solution was prepped by the earlier instructions from Eugene with all 3 isotopes at 100 ng/mL.

AMPA recovery is poor against the native standard with any matrix in the mix. AMPA IS corrects for that well, suppression makes sense since it isn't retained.

Chromatograms are attached if you'd like to look at the peaks.

Sensitivity came way back up after just cleaning the column with pure water and MeCN. Same mobile phase from earlier where we had the poor response and S/N. The column might need to be cleaned with solvent and re-conditioned with ion pairing reagents somewhat frequently as the sensitivity dropped over about 100 injections.

Thanks,

William "Bill" Cooke
Chemist

Office of Regulatory Affairs
Pacific Regional Laboratory Northwest
U.S. Food and Drug Administration
Office: (425) 487-5324

William.Cooke@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

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
Subject: Glyphosate proficiency
Date: Friday, February 10, 2017 2:08:00 PM

Hi Everyone,

Just a quick update about the progress of the glyphosate method lab proficiency results. To date I have received complete instrument proficiency data from ARL and PNW and partial data from NRL. The only method proficiency I have received came from PNW – see below. PNW calculated the AMPA with and without the IS, and the N-acetyl glyphosate with and without the glyphosate IS – see data below. Good Job, Bill! The AMPA obviously improves significantly with IS. The N-acetyl glyphosate improved slightly. Do we want to consider using the AMPA IS? Does everyone have the AMPA IS? Tell me what you think. It looks like carrot recoveries were slightly lower; it will be interesting to see if this is typical for all the labs.

We need to get the collab started. At this time LA and PNW are the only labs ready to collab. We need at least one more lab to provide both instrument and method proficiency data before we can begin.

Thanks and have a nice weekend,

Chris

	Glyphosate	ISTD	ESTD
Carrot		79.4	
Corn		98.0	
Avocado		93.3	
	Glufosinate		
Carrot		88.5	
Corn		97.5	
Avocado		91.7	
	AMPA		
Carrot		80.6	15.8
Corn		106.4	26.5
Avocado		96.1	17.4
	N acetylglphosate		
Carrot		91.9	72.6
Corn		100.5	90.0
Avocado		83.5	64.9

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
Subject: Update of instrument and method proficiency
Date: Tuesday, February 14, 2017 3:22:00 PM
Attachments: Collab-Glyphosate 2-7-16.xlsx

Hi Everyone,

Just want to give you a quick update of our progress with proficiency demonstration at each lab. I have received full instrument proficiency from 3 labs: ARL, PNW, and KAN. I have received partial instrument proficiency from NRL. Except for slightly elevated Lq for glufosinate at PNW, the instrument proficiency data indicates the LCMS method works exceptionally.

	ARL	PNW	NRL	KAN
	Glyphosate			
Lq (ng/ml)	0.2	0.4	0.2	0.5
Accuracy	100.3	98.4		100.3
Precision	6.3	2.8		1.2
Linearity	0.9970	0.9999		0.9999
	Glufosinate			
Lq (ng/ml)	0.3	4	0.1	0.6
Accuracy	99.8	96.2		100.2
Precision	1.9	0.7		0.6
Linearity	0.9999	0.9999		0.99999
	AMPA			
Lq (ng/ml)	0.2	2	0.3	0.3
Accuracy	100.5	96.4		100.2
Precision	11.9	3.3		1.6
Linearity	0.9985	0.9999		0.9999
	N acetylglphosate			
Lq (ng/ml)		6	0.3	
Accuracy		97.2		
Precision		6.7		
Linearity		0.9999		

I have received method proficiency data from only two labs: PNW and KAN. Both indicate the method works great.

	PNW*	PNW	KAN
	Glyphosate		
Carrot		79.4	104.8
Corn		98.0	101.5
Avocado		93.3	106.1

	Glufosinate			
Carrot			88.5	105.8
Corn			97.5	98.4
Avocado			91.7	100.6
	AMPA			
Carrot		80.6	15.8	52.3
Corn		106.4	26.5	29.3
Avocado		96.1	17.4	62.2
	N acetylglphosate			
Carrot		91.9	72.6	
Corn		100.5	90.0	
Avocado		83.5	64.9	

* IS used for AMPA and N-acetyl

Since we have 3 labs that have demonstrated both method and instrument proficiency, we can begin the collaboration. Before we start I would like everyone to review the attached draft collaboration protocol and let me know if we can finalize the protocol. If you are OK with the protocol, please send my an email indicating you recommend no changes. Otherwise, let me know any changes you recommend.

I hope we can start the collab in three labs this week.

Thanks,

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
Subject: Glyphosate collaboration
Date: Friday, February 17, 2017 8:04:00 AM

Just a quick update. LA provided instrument proficiency data – see below.

I would like to recommend a change to the glyphosate method calibration. We had agreed to use IS calibration for the parent compounds glyphosate and glufosinate, and external standard calibration for the degradants AMPA and N-acetylglyphosate. Because we are not accounting for the final volume of the extraction I think we should use (b) (5) for all analytes. Rather than messing with the IS isotopes for the degradants, I suggest we use the (b) (5) for them. Although the glyphosate IS will not account exactly for the recovery of the degradants, it will eliminate the volume of the extract as a variable. Recovery data from SEA using both ESTD and ISTD calibration of the degradants demonstrated a significant improvement for both when the ISTD calibration was used. This will also simply the calibration method so that all analytes are calibrated using IS calibration. Let me know what you think and I will update the collaboration to reflect this.

Thanks,

Chris

	ARL	PNW	NRL	KAN	SRL	PSW	Avg
	Glyphosate						
Lq (ng/ml)	0.2	0.4	0.2	0.5	0.2	0.3	0.3
Accuracy	100.3	98.4	101.4	100.3	99.3	99.1	99.9
Precision	6.3	2.8	1.6	1.2	0.5	1.4	2.5
Linearity	0.9970	0.9999	0.9999	0.9999	0.9997	0.9997	0.9993
	Glufosinate						
Lq (ng/ml)	0.3	4	0.1	0.6	1.5	0.3	1.3
Accuracy	99.8	96.2	101.4	100.2	98.9	99.8	99.3
Precision	1.9	0.7	4.7	0.6	1.0	2.3	1.8
Linearity	0.9999	0.9999	0.9996	0.99999	0.9995	0.9996	0.9998
	AMPA						
Lq (ng/ml)	0.2	2	0.3	0.3	0.3	0.5	0.62
Accuracy	100.5	96.4	105.1	100.2	98.8	97.7	100.2
Precision	11.9	3.3	2.2	1.6	1.0	2.1	4.0
Linearity	0.9985	0.9999	0.9988	0.9999	0.9991	0.9998	0.9992
	N acetylglphosate						
Lq (ng/ml)		6	0.3				3.2
Accuracy		97.2	102.1				99.7
Precision		6.7	5.5				6.1
Linearity		0.9999	0.9999				0.9999

From:	MacMahon, Shaun
To:	Sack, Chris A
Cc:	Noonan, Gregory
Subject:	RE: Glyphosate collab
Date:	Friday, February 17, 2017 12:00:16 PM
Attachments:	Sulfite SOP.docx Multi-laboratory Validation Plan Sulfites.docx MLV Proposal Review SUBMISSION FORM_v1.0 JNB.pdf

Hey Chris,

Attached are examples of a method SOP, MLV plan, and a completed proposal for your reference. Your spiking regime hits the requirement of at least 3 spiking concentrations in at least 3 matrices. Just a few questions: Why just the high spike in soy? Is it a recovery issue? Also, wasn't the method going to be applied to eggs and wheat? I suppose wheat and soy are similar enough, but eggs is a unique enough matrix that I could see including some spikes for that.

Happy to discuss any of this with you further.

Shaun

Shaun MacMahon, PhD
Phone: 240-402-1998

From: Sack, Chris A
Sent: Wednesday, February 15, 2017 12:46 PM
To: MacMahon, Shaun
Cc: Noonan, Gregory
Subject: Glyphosate collab

Hi Shaun,

We are almost ready for the glyphosate collab. The attached draft protocol has not been finalized by the PesTAG but it is close. I just wanted to run it by to see if you have any major concerns before we finalize. It is essentially the same protocol we discussed last August, just fleshed out a little. Also, I have the MLV application form you sent me. I will need some help with that.

Thanks,

Chris

From: Cooke, William
To: Sack, Chris A
Cc: Mercer, Gregory E; Mabry-Smith, Ronald C
Subject: RE: Glyphosate collaboration
Date: Friday, February 17, 2017 3:33:58 PM
Attachments: N-acetyl unscheduled.xlsx
N-acetyl.docx
N-acetyl 2.docx
Layout.PNG
Collaboration layout.cset
recoveries against ISTDs.xlsx
image003.png

Hi Chris,

In the earlier recovery data I had used AMPA IS for the internal standard. I reprocessed the AMPA data using ESTD, AMPA IS, Glufo IS, and Glypho IS (attached). The suppression on AMPA is only addressed with AMPA IS as Glufosinate-D3 and Glyphosate 13C2 15N elute too much later.

Had some time yesterday and tuned on n-acetyl glyphosate. The 6500 had better response dropping the DP down to 20 from Eugene's 88 DP. Found a few other transitions that had better S/N. I ran them unscheduled on our spikes with both our conditions and Eugene's for the first 3 transitions (210-63, 210-124, 210-79) with only the DP changed. The lower DPs had better response on the 6500.

Of the additional 3 transitions, 210 – 168 and 210 – 150 worked well with good S/N and minimal interferences. 210 -192 (loss of water) was very noisy. 210-168 is a loss of 42, corresponds to –COCH2 and fits losing the acetyl group. 210-150 is loss of 60, corresponds to –COCH2 and –H2O. Running the standards in MS/MS/MS using the ion trap confirmed that breakdown pathway (210 can break into 168 and then 150). Further digging showed the new transitions can fragment down to the 63 (PO2-) and 79 (PO3-) fragments.

My N-acetyl conditions are

Q1	Q2	RT	Transition	DP	EP	CE	CXP
210	63	4.4	N-acetyl glyphosate 1	-20	-11	-40	-13
210	124	4.4	N-acetyl glyphosate 2	-20	-11	-17	-13
210	79	4.4	N-acetyl glyphosate 3	-20	-11	-50	-13
210	150	4.4	N-acetyl glyphosate 4	-20	-11	-20	-13
210	168	4.4	N-acetyl glyphosate 5	-20	-11	-18	-13

Looking at this run, I'd retag the transitions as

Q1	Q2	RT	Transition	DP	EP	CE	CXP
210	150	4.4	N-acetyl glyphosate 1	-20	-11	-20	-13
210	63	4.4	N-acetyl glyphosate 2	-20	-11	-40	-13
210	168	4.4	N-acetyl glyphosate 3	-20	-11	-18	-13

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: Cassias, Irene; Eide, David J; Katsoudas, Eugenia; MacMahon, Shaun; Sack, Chris A; Podhorniak, Lynda
Subject: Glyphosate collaboration
Date: Monday, February 20, 2017 12:15:00 PM
Attachments: Collab-Glyphosate Final.xlsx
SEA Layout 2-17-17.PNG
SEA Collaboration layout 2-17-17.cset
Glyphosate method Collab Final.docx
SEA N-acetyl 2 2-17-17.docx

Hi Everyone,

Bill Cooke did some work with N-acetyl glyphosate on the 6500 and found two new transitions that work better than those in the method.

Q1	Q2	RT	Transition	DP	EP	CE	CXP
210	150	4.4	N-acetyl glyphosate 1	-20	-11	-20	-13
210	63	4.4	N-acetyl glyphosate 2	-20	-11	-40	-13
210	168	4.4	N-acetyl glyphosate 3	-20	-11	-18	-13

The data and chromatograms he provided (see attached file "SEA N-acetyl 2 2-17-17.docx") clearly demonstrate the advantages of changes to the LC-MS/MS parameters. I have inserted these changes in the final method and collaboration protocol that are attached. Note I highlighted the changes in red. Note also, that I changed the transition names in the calibration method for the collab protocol – those changes are in red also. I would like everyone to try these parameters and verify they work for your instrument. Please note the DP voltage for the 5500 might be optimized at much larger levels.

Bill also analyzed some spikes using various IS calibrants for AMPA and N-acetyl glyphosate. The results tabulated below clearly indicate the benefit of using the glyphosate-13C IS for the calibration N-acetyl glyphosate. For AMPA Bill compared all three available IS isotopes. Obviously, the AMPA isotope works best, but we have already decided we will not be quantitating AMPA. The glyphosate IS appears to work satisfactorily to compensate the sample volume differences between matrices. I updated the collab protocol to use glyphosate-13C as an IS for glyphosate, AMPA, and N-acetyl glyphosate and glufosinate-D3 for glufosinate. These changes are in red also.

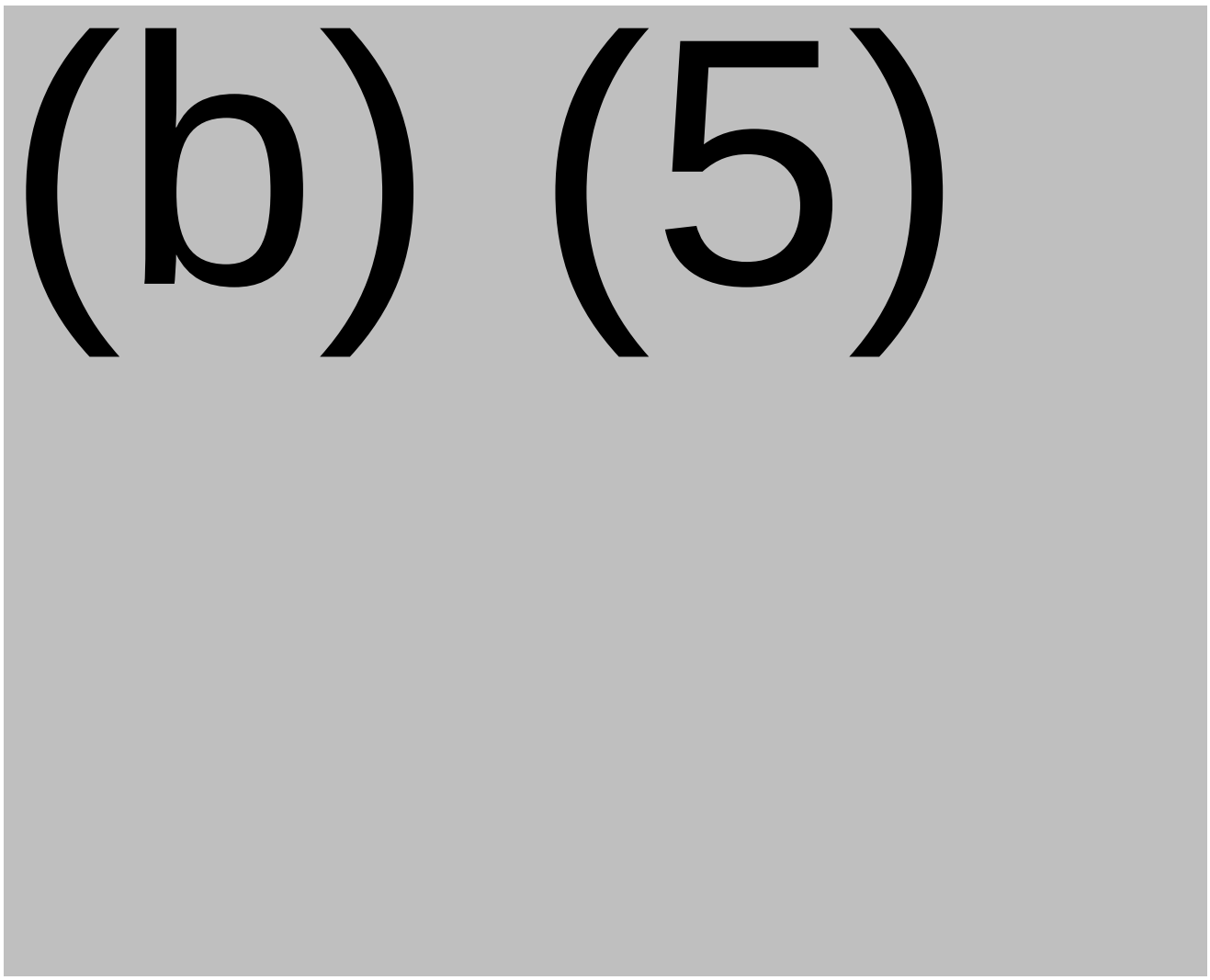
	AMPA Spike 200				**N-acetyl glyphosate Spike 200**	
IS	**AMPA**	**Glyphosate**	**Glufosinate**	**None**	**Glyphosate**	**None**
Avocado	96	22	52	17	84	65
Carrot	81	20	29	16	92	73
Corn	106	30	32	26	100	90

When I was with Bill last week, I asked him to provide me a results file formatted as directed in the collab protocol. He provided a screen shot "SEA Layout 2-17-17.png" – see attached. In his example Bill has provided all the data fields listed in the protocol along with a few extras, including Height, Ion Ratio, Accuracy, Mass Info and Area Ratio. This format is fine with me. As long as the transition masses are correct in the transition name, the Mass Info data is redundant. The other

extra fields could prove useful but are not necessary.

Collab protocol	**SEA example**
Index	Index
Sample Name	Sample Name
Sample Type	Sample Type
Dilution Factor	Dilution Factor
Peak Name (Transition Name)	Component Name
Peak Area	Area
IS Peak Area	IS Area
RT	Retention Time
Concentration (Spk level or Std conc)	Actual Concentration
Calc concentration	Calculated concentration

Some notes and observations:



Thanks everyone,

Chris

From: Sack, Chris A
To: Thompson, Richard L.; Chang, Eugene; Cooke, William
Cc: Mercer, Gregory E
Subject: PE vs no PE
Date: Tuesday, February 21, 2017 8:17:00 AM

Hi Eugene, Richard, and Bill,

In the method I have indicated the PE cleanup is optional for fatty or dirty matrices. I forgot to include instructions in the collab protocol. What do you guys think? I was assuming everyone would use the PE cleanup for the avocado. Should I include analyses with and without PE cleanup for corn and carrot? I don't want some QA guy questioning the option down the road. If it was up to me I would add the PE cleanup to all analyses for the sake of simplicity and the extra cleanup probably wouldn't hurt recoveries of such polar analytes.

What do you think?

Chris