Method development/validation of the direct determination of glyphosate, glufosinate, and AMPA in Food by LC/MS.

Narong Chamkasem, ***Southeast Regional Laboratory, U.S. Food and Drug Administration, Atlanta, GA***

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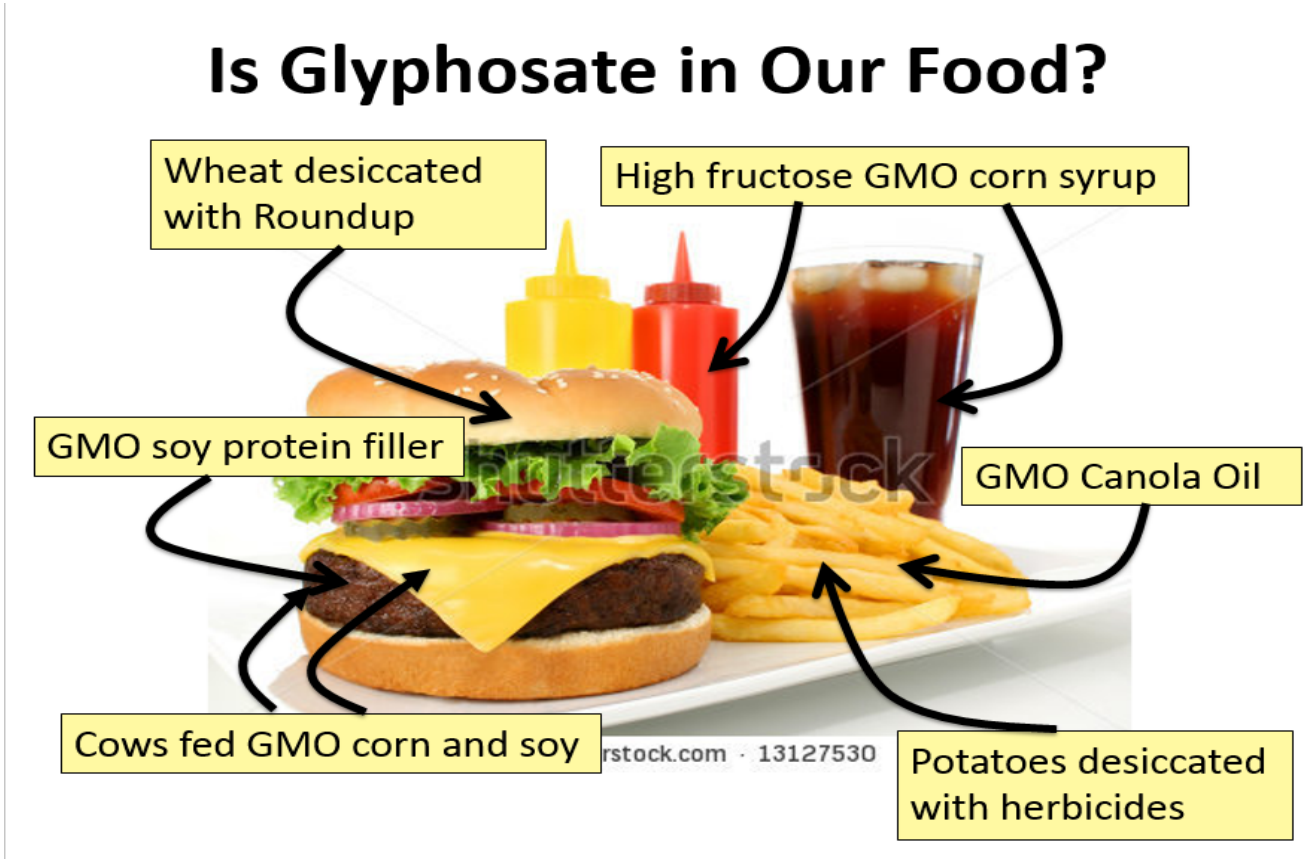
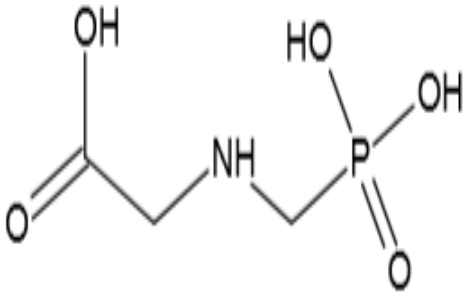
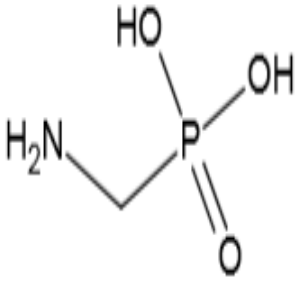
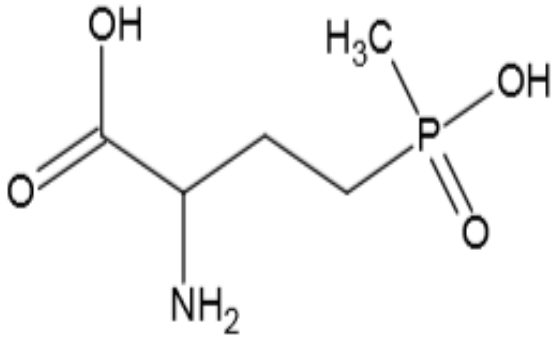




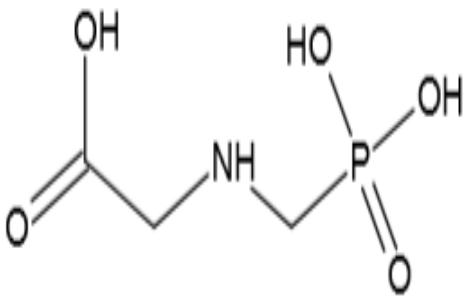
Glyphosate



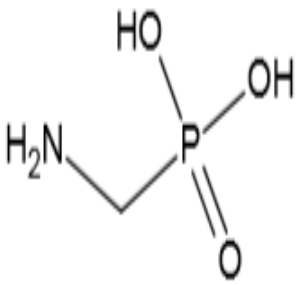
AMPA



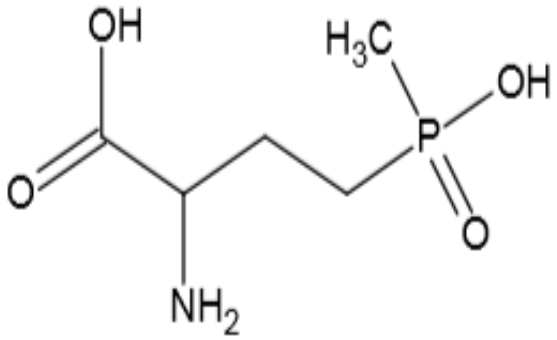
Glufosinate



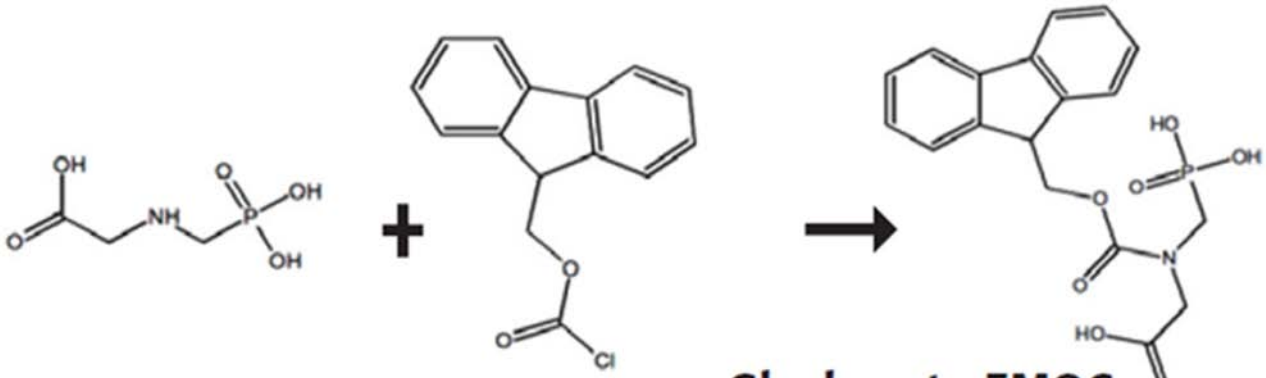
Glyphosate



AMPA



Glufosinate



Glyphosate + FMOC-Cl → Glyphosate-FMOC
(Monoisotopic mass = 391)

FMOC-Cl = Fluorenylmethyloxycarbonyl chloride

Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPe-Method)

- **Version 7.1** (Nov 2013, Document History, see page 434)

Authors: M. Anastassiades; D. I. Kolberg; D. Mack; C. Wildgrube; I. Sigalov; D. Dörk

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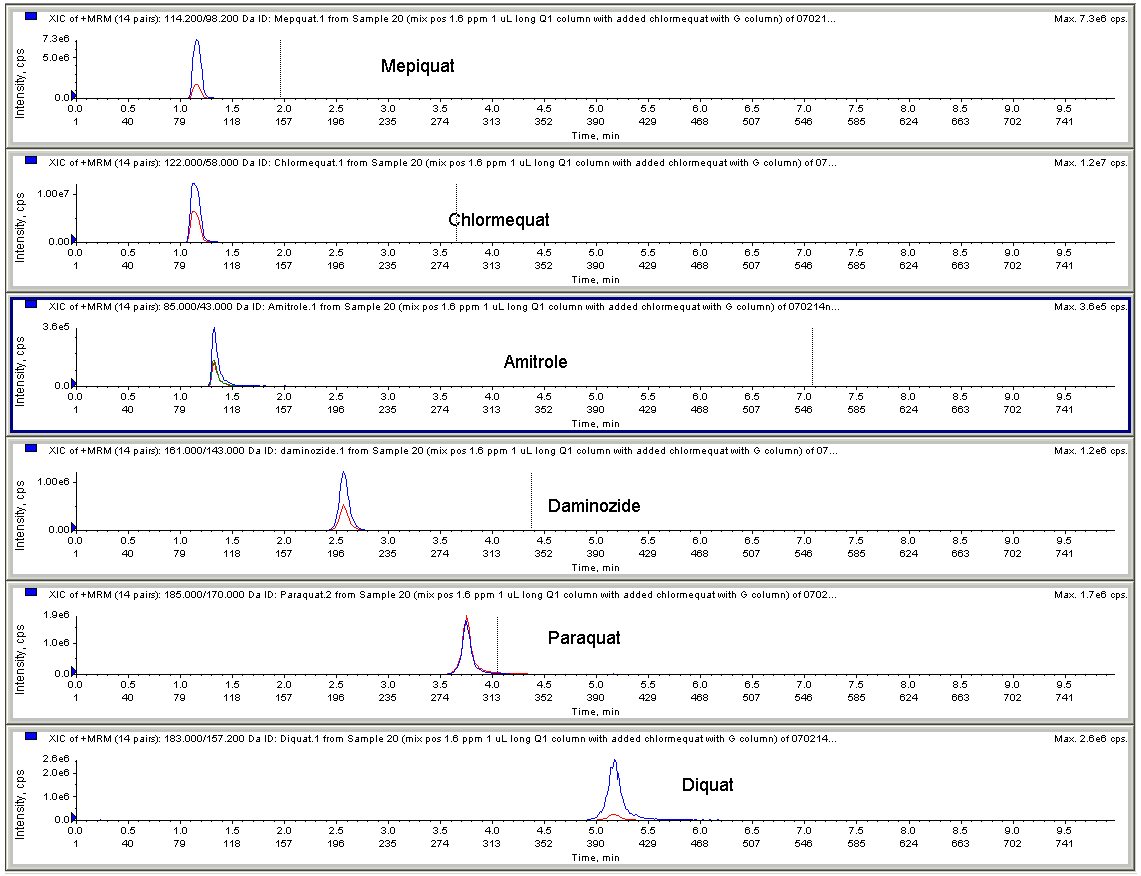
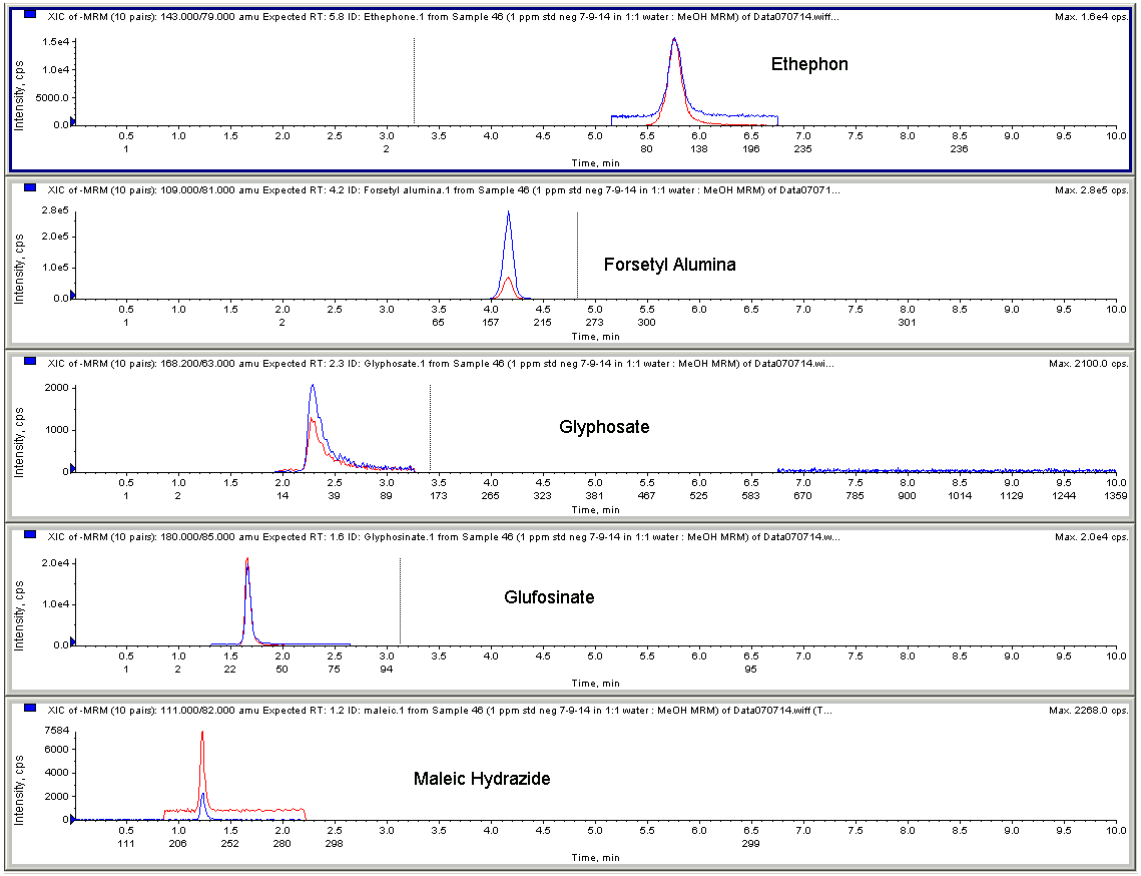
	M 1.1	M 1.2	M 1.3	M 2	M 3	M 4
	Anion Exchange (AS-11)	Anion Exchange (AS11-HC)	Carbon (Hypercarb)	Normal Phase (Obelisc-R)	Normal Phase (Obelisc-R)	Normal Phase (Obelisc-R)
Ethephon	✓	✓	✓			
HEPA	✓	✓	✓			
Glufosinate	✓	✓	✓			
N-Acetyl-Glufosinate	✓	✓	✓			
MPPA	✓	✓	✓			
Glyphosate	✓	✓	✓			
AMPA	✓	✓	✓			
Phosphonic acid	✓	✓	✓			
N-Acetyl-AMPA		✓	✓			
Fosetyl-Al		✓	✓	✓		
Maleic hydazide			✓	✓		
Perchlorate			✓	✓		
Chlorate			✓			
Amitrole					✓	
ETU					✓	
PTU					✓	
Cyromazin					✓	✓
Trimesium					✓	✓
Daminozide					✓	✓
Chlormequat					✓	✓
Mepiquat					✓	✓
Difenzoquat					✓	✓
Diquat						✓
Paraquat						✓

Polar analyte by LC/MS in a positive mode



Polar analyte by LC/MS in a negative mode



Glyphosate method in milk

Sample extraction procedure

1 Pipette 1 g milk into a 15-mL tube

2 Add 3 mL Extracting solvent (50 mM acetic acid/10 mM Na_2EDTA)

3 Shake 10 min (2000 stroke/min) on a Geno grinder and centrifuge at 3000 rpm for 5 min

4 Passing 2 mL of the top layer through a 60 mg/3 mL Oasis HLB (no conditioned needed)

5 Mix the sample with IS and inject to LC/MS

HPLC condition:

Column - Trinity Acclaim Q1 (100 x 3 mm, 3 um), with a C18 SecurityGuard (4 x 3 mm), 10 uL

Mobile phase: 50 mM ammonium formate (pH 2.9) at 0.5 mL/min, 35 C, 6 min,

MS condition: AB Sciex 5500 (Q-Trap), Ion-spray neg-mode, source temp 350 C,

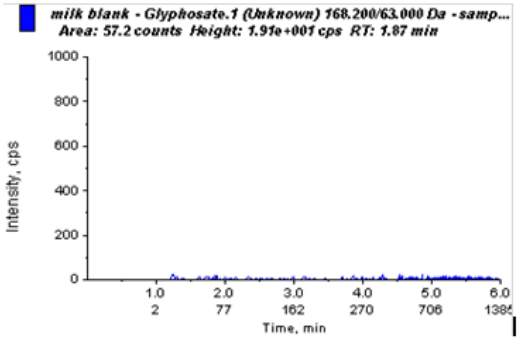
Analyte	Precursor Ion (m/z)	Product Ion (m/z)	DP	CE	EP	CXP	Retention Time (min)
AMPA.1	110	63	-60	-24	-10	-10	1.1
AMPA.2	110	79	-60	-26	-10	-10	1.1
AMPA $^{13}C^{15}N$ (IS)	112	63	-60	-24	-10	-10	1.1
Glufosinate.1	180	95	-46	-23	-10	-10	1.65
Glufosinate.2	180	85	-46	-26	-10	-10	1.65
Glufosinate D3 (IS)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

Results

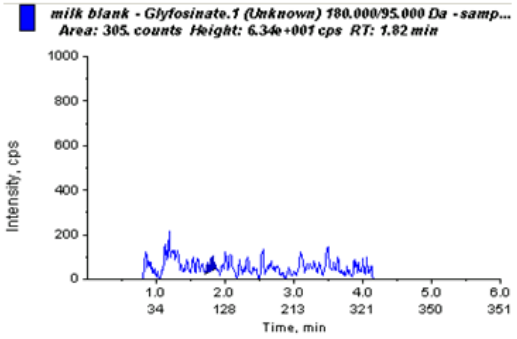
	Fortification level		Matrix with IS	Matrix without IS	Solvent with IS	Solvent without IS
Glyphosate	25 ng/mL	Recovery (%)	81	85	**85**	**123**
		RSD (%)	8.36	9.94	7.62	7.3
	100 ng/mL	Recovery (%)	107	110	**111**	**106**
		RSD (%)	2.78	2.76	2.19	3.25
	500 ng/mL	Recovery (%)	93	99	**100**	**98**
		RSD (%)	2.04	2.41	2.05	2.37
	2000 ng/mL	Recovery (%)	91	97	**99**	**97**
		RSD (%)	3.36	3.15	4.54	3.16
Glufosinate	25 ng/mL	Recovery (%)	86	89	**90**	**123**
		RSD (%)	5.43	6.83	7.66	8
	100 ng/mL	Recovery (%)	106	106	**105**	**96**
		RSD (%)	3.62	3.62	4	4.94
	500 ng/mL	Recovery (%)	94	101	**96**	**93**
		RSD (%)	6.01	1.64	6.16	1.52
	2000 ng/mL	Recovery (%)	94	99	**97**	**92**
		RSD (%)	7.07	1.31	7.04	1.38
AMPA	25 ng/mL	Recovery (%)	83	89	**84**	**43**
		RSD (%)	2.73	5.12	3.86	2.35
	100 ng/mL	Recovery (%)	104	115	**106**	**28**
		RSD (%)	6.65	2.98	7.06	3.28
	500 ng/mL	Recovery (%)	90	113	**97**	**17**
		RSD (%)	4.35	2.53	5.44	2.67
	2000 ng/mL	Recovery (%)	93	113	**100**	**15**
		RSD (%)	5.17	2.26	6.06	2.62

Milk blank

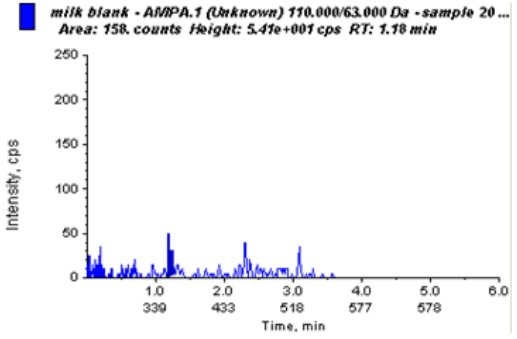
Glyphosate channel



Glufosinate channel

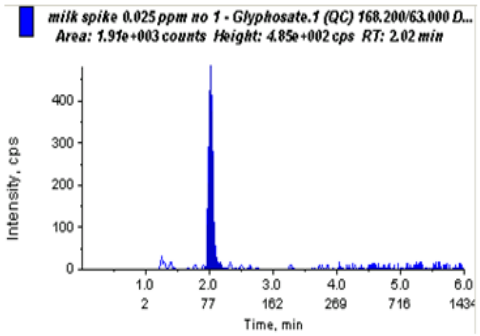


AMPA channel

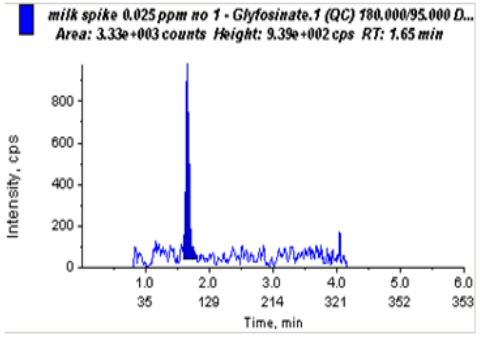


Milk blank fortified at 25 ng/g of glyphosate, glufosinate and AMPA

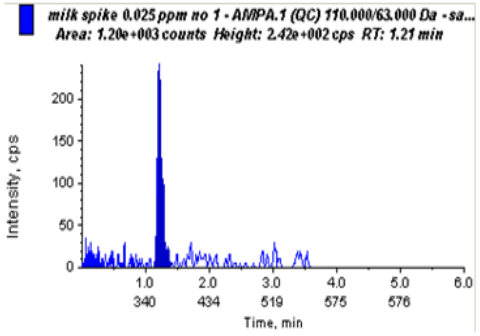
Glyphosate channel



Glufosinate channel



AMPA channel



Glyphosate method in soybean/corn

1 Glyphosate was the most applied chemical in world (180-185 million lbs. in US during 2007)

2 The use of glyphosate has increased significantly with roundup soybean and corn

3 Has high tolerance (20 ppm for soybean and 10 ppm corn)

4 No method available for regulatory action.
GAO audit in 2014, so FDA must screen it for public safety.

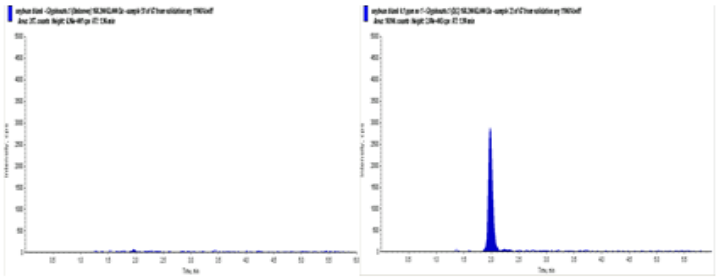
Sample extraction procedure

Soybean/Corn method

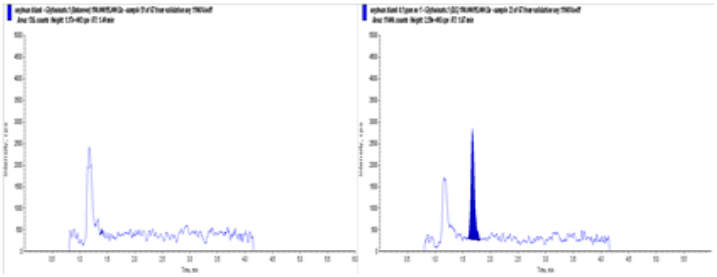
1. 2 g sample in a 50-mL tube + 10 mL extracting solvent
2. Shake for 10 min and centrifuge for 5 min
3. Pass 2 mL to a 60 mg/3 mL Oasis HLB SPE
4. Mix the extract with IS and inject to LC/MS

Chromatogram of soybean blank (left) and soybean blank fortified at 0.1 µg/g of glyphosate, glufosinate and AMPA (right)

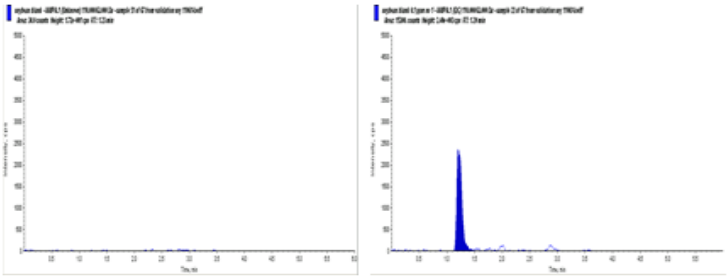
Glyphosate channel



Glufosinate channel

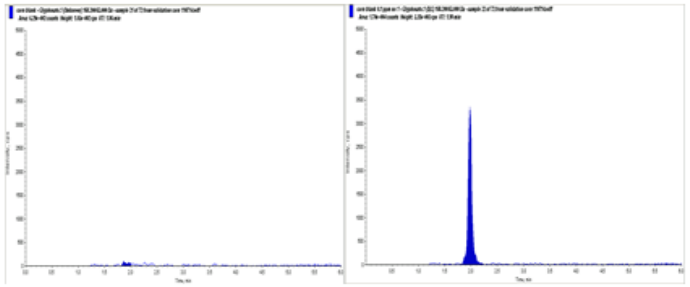


AMPA channel

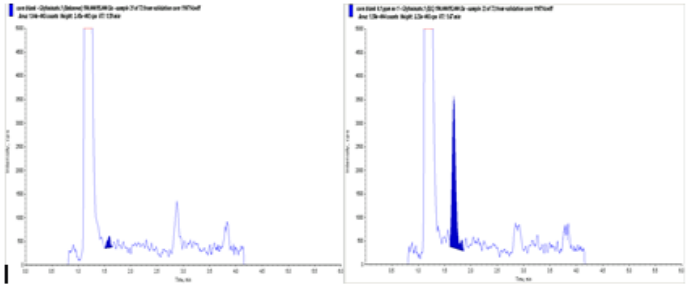


Chromatogram of corn blank (left) and corn blank fortified at 0.1 µg/g of glyphosate, glufosinate and AMPA (right)

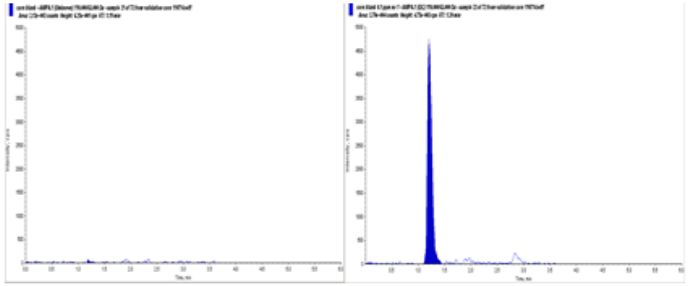
Glyphosate channel



Glufosinate channel



AMPA channel



Soybean

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

Corn

Corn (n = 7)

Analyte	Fortification level (ug/g)		Calibration method: Matrix with IS	Matrix no IS	Solvent with IS	Solvent no IS
Glyphosate	0.1	Recovery (%)	100	89	104	105
		RSD (%)	4.8	6.3	3.6	5.4
	0.5	Recovery (%)	104	96	104	99
		RSD (%)	4..2	4.0	4.2	3.9
	2	Recovery (%)	107	97	106	98
		RSD (%)	3.8	2.7	3.8	2.8
Glyfosinate	0.1	Recovery (%)	92	96	99	97
		RSD (%)	8.6	9.9	4.8	9.1
	0.5	Recovery (%)	103	99	107	94
		RSD (%)	3.9	3.7	3.7	3.6
	2	Recovery (%)	103	99	101	92
		RSD (%)	5.3	3.4	5.3	3.3
AMPA	0.1	Recovery (%)	96	NA	113	NA
		RSD (%)	11.9	NA	6.5	NA
	0.5	Recovery (%)	103	8.2	111	NA
		RSD (%)	8.3	48.6	7.8	NA
	2	Recovery (%)	105	52	110	104
		RSD (%)	6.9	5.8	6.9	9.3

NA = not applicable

Sample extraction procedure

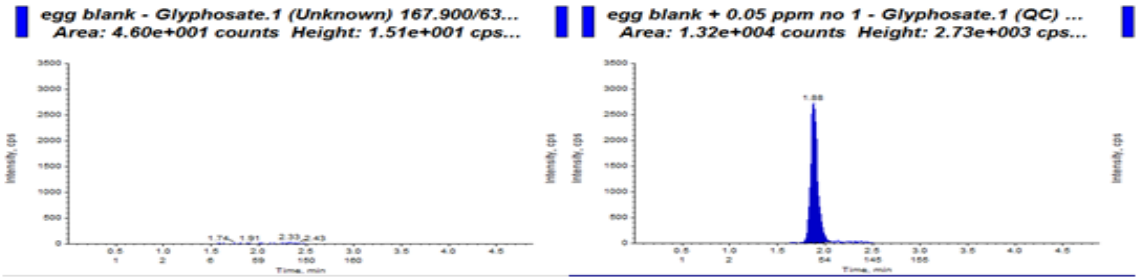
Egg method

1. 2 g sample in a 50-mL tube + 8.5 mL solvent and 6 mL methylene chloride
2. Shake 10 min and centrifuge for 5 min
3. Pass 2-3 thru a Whatman 0.2 µm GD/X Nylon filter into a 60 mg/3 mL Oasis HLB SPE
4. Mix the extract with IS and inject to LC/MS

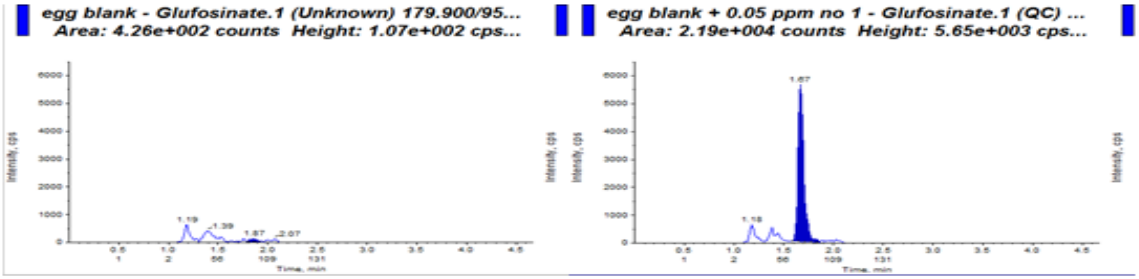
Issue in egg sample

Chromatogram of egg blank (left) and egg blank fortified at 0.05 µg/g of AMPA, glufosinate, and glyphosate

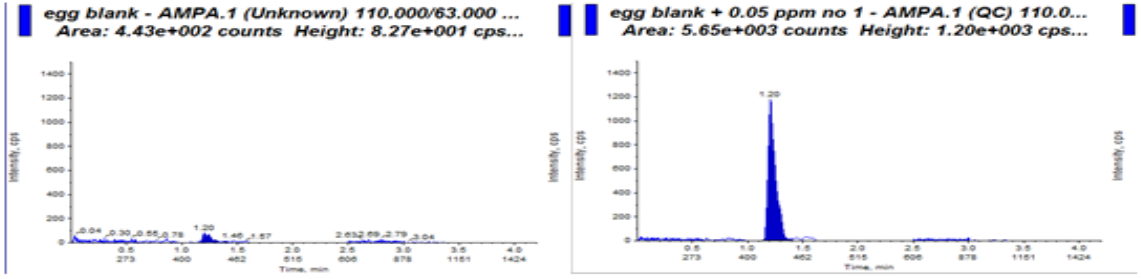
Glyphosate channel



Glufosinate channel



AMPA channel



Egg

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

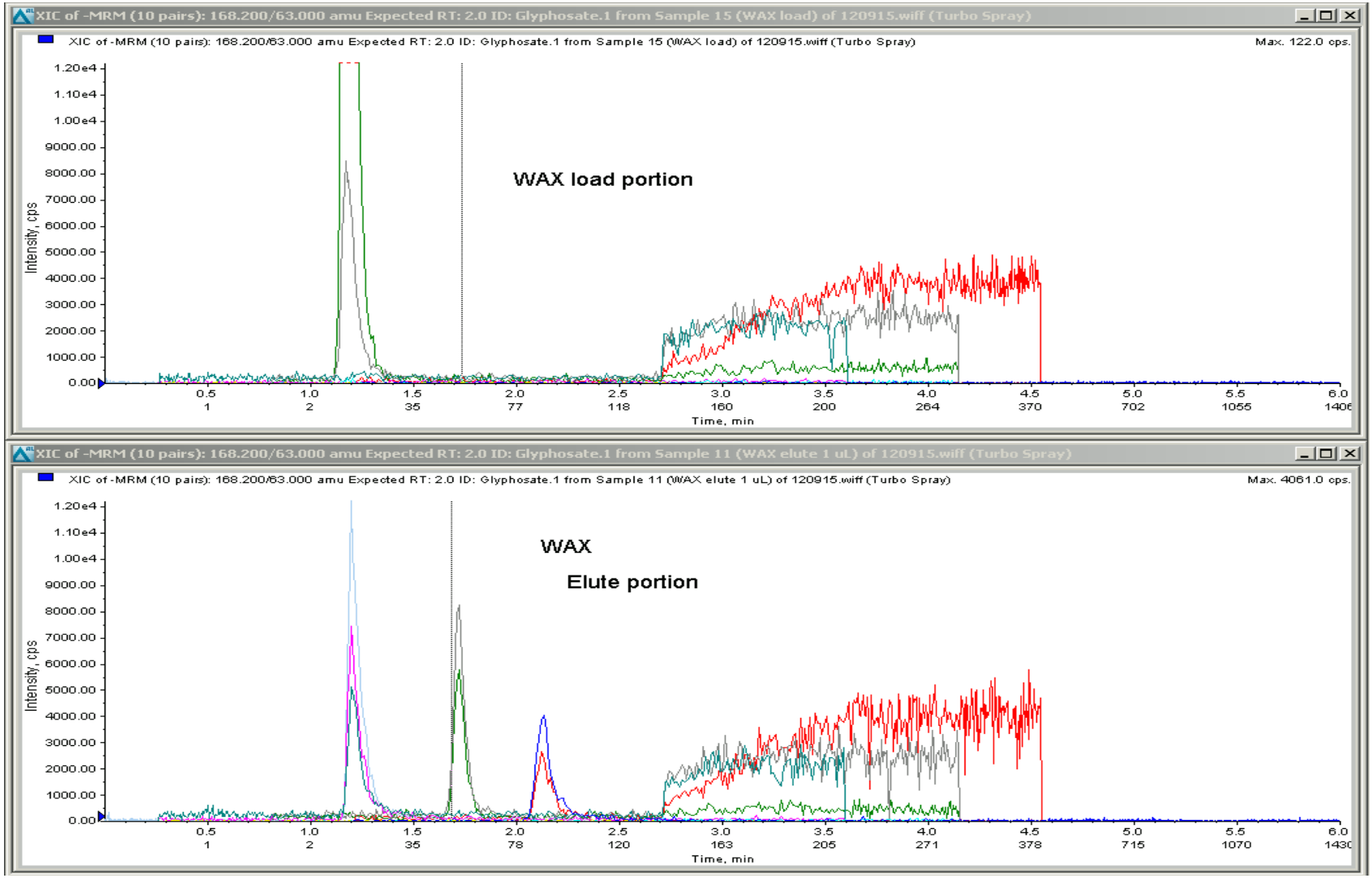
JANUARY 20, 2016
SUSTAINABLE PULSE

Pennsylvania Researchers Discover Glyphosate Herbicide in Honey and Soy Sauce

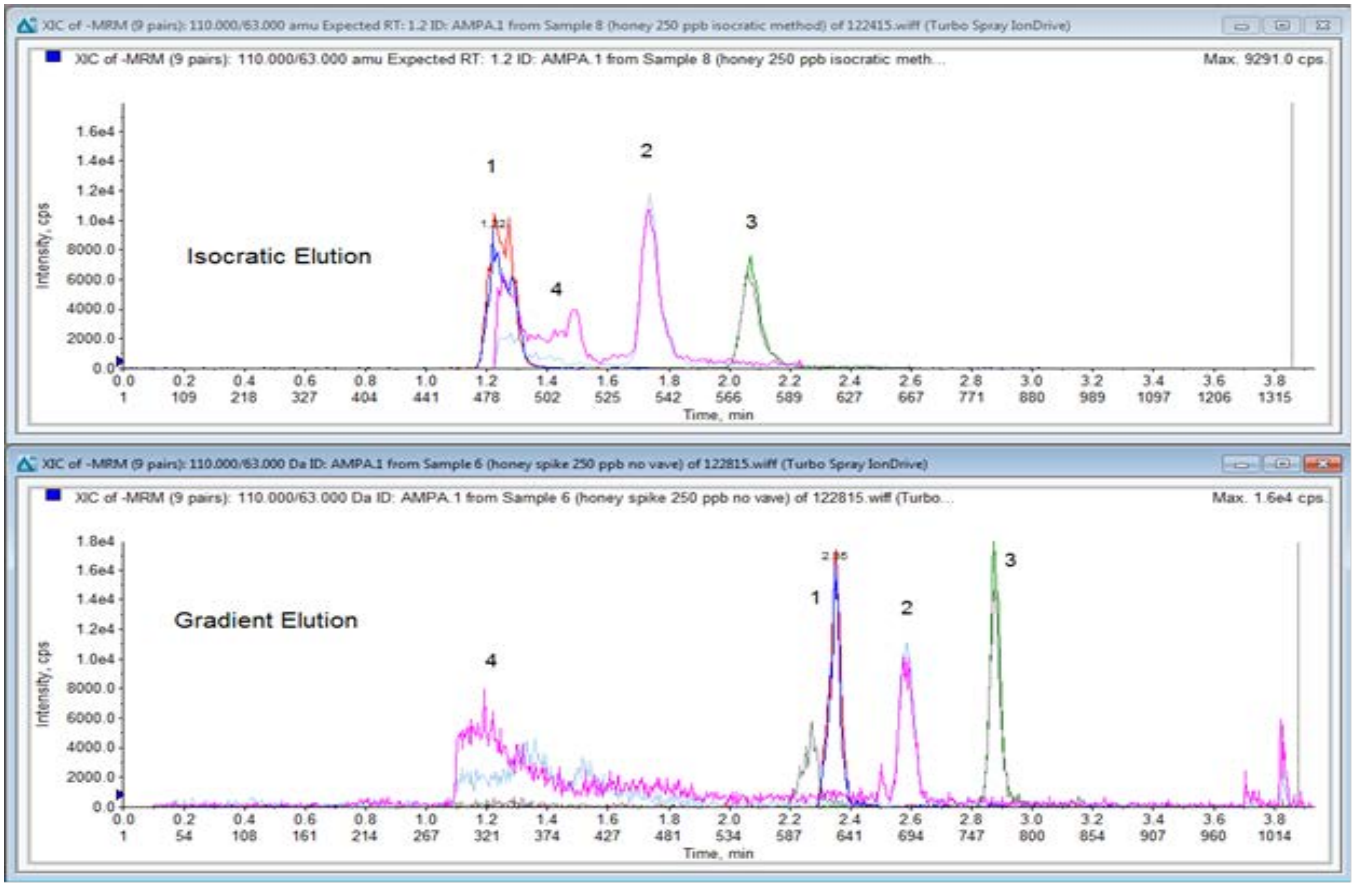
Of the sixty-nine honey samples analyzed, forty-one samples, or fifty-nine percent (59%), had glyphosate concentrations above the method LOQ (15 ppb), with a concentration range between 17 and 163 ppb and a mean of 64 ppb.

Even more surprisingly five of the eleven organic honey samples, or forty-five percent (45%), contained glyphosate concentrations above the method LOQ, with a range of 26 to 93 ppb and a mean of 50 ppb.

WAX cleanup procedure



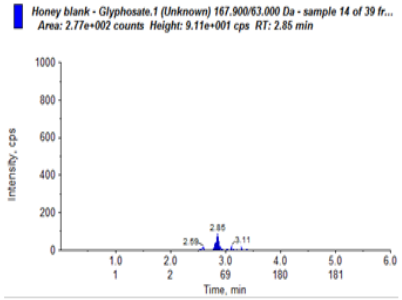
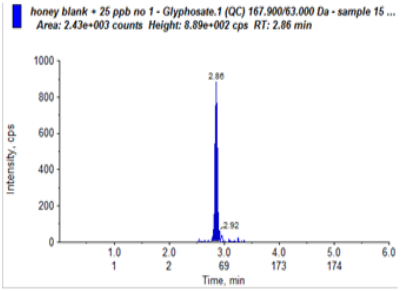
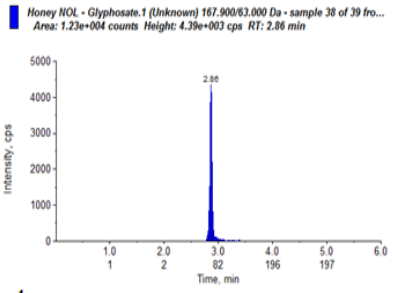
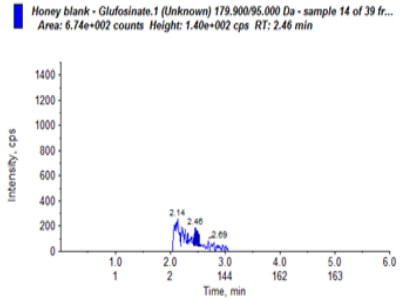
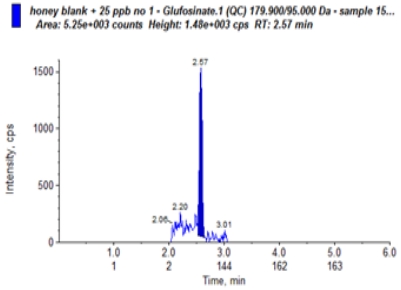
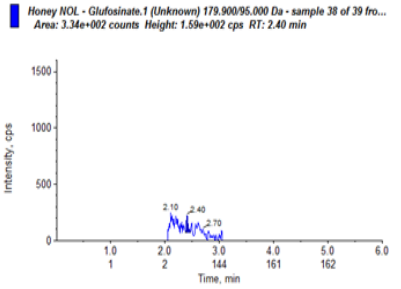
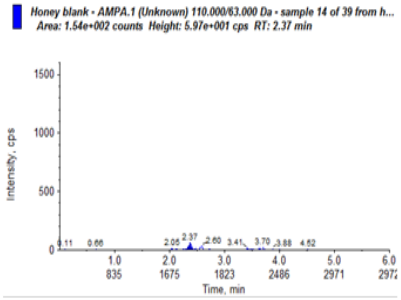
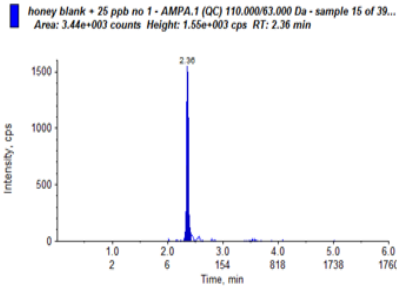
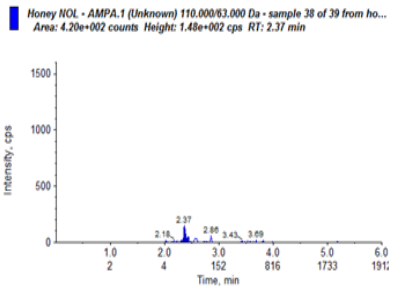
Isocratic vs. Gradient elution



1 = AMPA, 2 = Glufosinate, 3 = Glyphosate, 4 = honey matrix

Analyte	Fortification level (ng/g)	Recovery (%)	RSD (%)	LOD ng/g	LOQ ng/g	Linearity R square
Honey A, honey from Ivory Coast, (n = 4)						
Glyphosate	25	92	7.0	5	16	0.9997
	50	102	2.1			
	100	92	2.6			
	500	96	2.8			
Glufosinate	25	107	6.3	5	17	0.9991
	50	107	5.3			
	100	91	3.0			
	500	98	2.7			
AMPA	25	106	1.6	1	4	0.9998
	50	104	3.0			
	100	90	3.4			
	500	103	3.1			
Honey B [a], organic honey. (n = 7)						
Glyphosate	25	90	12.1	8	26	0.9981
	50	93	4.6			
	100	87	8.0			
	500	102	8.1			
Glufosinate	25	107	6.8	5	18	0.9990
	50	94	5.9			
	100	90	8.2			
	500	101	7.3			
AMPA	25	111	5.6	5	16	0.9994
	50	103	3.5			
	100	93	6.7			
	500	103	3.5			

a) Honey B contains 10 ng/g glyphosate

Honey blank	Honey blank + 25 ng/g	Honey containing 121 ng/g Glyphosate
Glyphosate channel	Glyphosate channel	Glyphosate channel
		
Glufosinate channel	Glufosinate channel	Glufosinate channel
		
AMPA channel	AMPA channel	AMPA channel
		

Glyphosate found in honey samples collected from the local market

Sample	glyphosate found (ng/g) [a]	Source
wild flower honey	trace	GA, USA
organic honey	17	Brazil
orange blossom honey	trace	FL, USA
clover honey	26	GA, USA
orange blossom honey	21	USA
clover honey	40	USA
clover honey	trace	Canada
wild flower honey/miel	46	Canada
Bonne Maman honey	trace	unknown
Manuka Honey	trace	New Zealand
honey	trace	France
Billy bee honey	19	Canada
honey	trace	Ivory Coast
honey blend with fructose/flavor	trace	Taiwan
local honey	trace	GA, USA
organic honey	trace	TX, USA
honey from FL	24	FL, USA
honey from LA	121	LA, USA
honey from IA	35	IA, USA

a trace = amount found less than 16 ng/g (estimated LOQ)

TECH & SCIENCE

THE FDA WILL BEGIN TESTING FOOD FOR GLYPHOSATE, THE MOST HEAVILY USED FARM CHEMICAL EVER

BY ZOË SCHLANGER ON 2/19/16 AT 6:18 PM

THE BLOG

Private Tests Show Cancer-Linked Herbicide in Breakfast Foods; FDA mum on Its Assessments

04/19/2016 09:12 am ET | **Updated** Apr 19, 2016

Carey Gillam
Veteran journalist; Research Director for U.S. Right to Know, a non-profit consumer education group

And I found these.

	glyphosate	AMPA
	ppm	ppm
Apple Cinnamon instant oat meal	0.01	0.01
Maple Brown Sugar instant oat meal	1.24	0.07
Cinnamon Spice instant oat meal	1.67	0.07
Peach and Cream instant oat meal	0.36	0.01
soy milk	0.00	0.00
Q steel cut oats (not instant)	1.53	0.07
Infant oat cereal	1.56	0.07
Infant oat banana	0.30	0.04
Infant oatmeal banana strawberry	0.83	0.08
Whole grain oat cereal	0.03	0.01
organic oats Bob' Red mill	ND	ND
organic oats 365 Whole Foods	ND	ND
organic oats Sprout	ND	ND
organic oats Nature Path	ND	ND
NC's cereal with oats and wheat	0.20	ND

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Regulatory Science

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Direct Determination of 2,4-dichlorophenoxyacetic acid in Soybean and Corn by Liquid chromatography/tandem mass spectrometry

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

[a]*Southeast Regional Laboratory, U.S. Food and Drug Administration, 60 Eighth Street, N.E., Atlanta, GA, 30309*

Combine glyphosate method into one to include high moisture samples (fruit-vegetable) by mixing sample with solvent-methylene chloride / Oasis HLB and use step gradient.

Direct determination of Paraquat/Diquat in potatoes by LC/MS


Thank You