MultiQuant - [[MQ4] Results Table (20170202 Filters.qsession)]

File Edit Process Window Help

Glyphosate

Components & Groups | IS

All Components

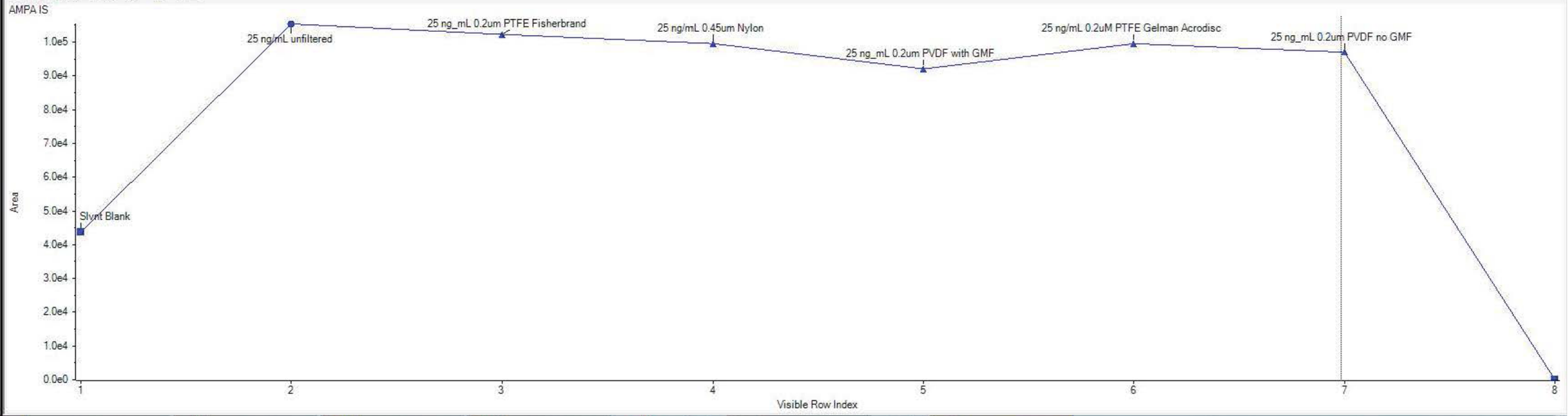
AMPA Group
IS Group
Glufosinate Group
Glyphosate Group
N-acetyl glyphosate Group

All Internal Standards
AMPA IS
Glufosinate IS
Glyphosate IS

All Analytes
AMPA 1
AMPA 2
Glufosinate 1
Glufosinate 2
Glufosinate 3
Glyphosate 1
Glyphosate 2
Glyphosate 3
N-acetyl glyphosate 1
N-acetyl glyphosate 2
N-acetyl glyphosate 3

All Sample Types

Index	Sample Name	Sample Type	Dilution Factor	Component Name	Outlier Reasons	Actual Concentration	Area	Area Ratio	Height	Retention Time	Used	Calculated Concentration	Ion Ratio	Accuracy
3	Slvnt Blank	Unknown	1.00	AMPA IS		1.00	43725	N/A	5110	1.30		N/A	1.0000	N/A
17	25 ng/mL unfiltered	Standard	1.00	AMPA IS		1.00	105377	N/A	9217	1.33		N/A	1.0000	N/A
31	25 ng_mL 0.2um PTFE Fisherbrand	Quality Control	1.00	AMPA IS		1.00	102293	N/A	8806	1.33		N/A	1.0000	N/A
45	25 ng/mL 0.45um Nylon	Quality Control	1.00	AMPA IS		1.00	99571	N/A	8648	1.32		N/A	1.0000	N/A
59	25 ng_mL 0.2um PVDF with GMF	Quality Control	1.00	AMPA IS		1.00	92080	N/A	8227	1.32		N/A	1.0000	N/A
73	25 ng/mL 0.2uM PTFE Gelman Acrodisc	Quality Control	1.00	AMPA IS		1.00	99541	N/A	8370	1.33		N/A	1.0000	N/A
87	25 ng_mL 0.2um PVDF no GMF	Quality Control	1.00	AMPA IS		1.00	97001	N/A	8038	1.33		N/A	1.0000	N/A
101	H2O	Unknown	1.00	AMPA IS		1.00	N/A	N/A	N/A	N/A		N/A	N/A	N/A



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

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, 150 x 4.6 mm, 5 µm, Phenomenex 00F-4040-E0 (pic attached)
    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
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

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Filter, 2 µm, 25 mm,
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 200-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

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

C. Standard Solutions

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

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

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

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

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

7. Sample concentration: 0.2 g/ml

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

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

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP	EP	CE	CXP
110	63	2.5	AMPA 1	-40	-11	-30	-9
110	79	2.5	AMPA 2	-40	-11	-34	-9
110	81	2.5	AMPA 3	-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	-19	-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

(b) (5)

MS Parameters

CUR (b) (5)

CAD MEDIUM

FDA Glyphosate Method

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

Level (ng/g)	Spk Added (ng)	Spk Std Conc (µg/ml)	Spk Vol Added (µl)	Std Dilution* (ml)	Cal Std Conc (ng/ml)
	Corn		*2 g*		
50	100	1	100	10	10
200	400	5	80	10	40
500	1000	5	200	10	100
Carrot/Avocado			*5 g*		
50	250	5	50	25	10
200	1000	5	200	25	40
500	2500	50	50	25	100

*Dilute with control matrix extract

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Glufosinate 3
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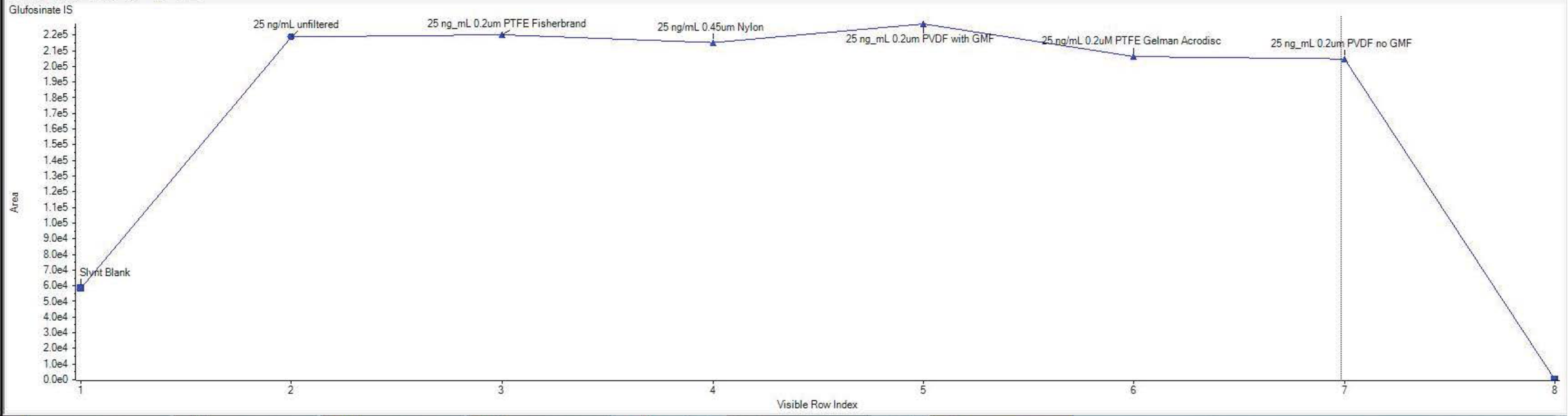
Glyphosate 3
N-acetyl glyphosate 1
N-acetyl glyphosate 2
N-acetyl glyphosate 3

All Sample Types

Index	Sample Name	Sample Type	Dilution Factor	Component Name	Outlier Reasons	Actual Concentration	Area	Area Ratio	Height	Retention Time	Used	Calculated Concentration	Ion Ratio	Accuracy
7	Slvnt Blank	Unknown	1.00	Glufosinate IS		1.00	58358	N/A	6146	2.69		N/A	1.3347	N/A
21	25 ng/mL unfiltered	Standard	1.00	Glufosinate IS		1.00	218774	N/A	19324	3.31		N/A	2.0761	N/A
35	25 ng_mL 0.2um PTFE Fisherbrand	Quality Control	1.00	Glufosinate IS		1.00	220180	N/A	19654	3.31		N/A	2.1525	N/A
49	25 ng/mL 0.45um Nylon	Quality Control	1.00	Glufosinate IS		1.00	215156	N/A	19409	3.31		N/A	2.1608	N/A
63	25 ng_mL 0.2um PVDF with GMF	Quality Control	1.00	Glufosinate IS		1.00	227071	N/A	18839	3.55		N/A	2.4660	N/A
77	25 ng/mL 0.2uM PTFE Gelman Acrodisc	Quality Control	1.00	Glufosinate IS		1.00	206237	N/A	18226	3.32		N/A	2.0719	N/A
91	25 ng_mL 0.2um PVDF no GMF	Quality Control	1.00	Glufosinate IS		1.00	204722	N/A	18059	3.31		N/A	2.1105	N/A
105	H2O	Unknown	1.00	Glufosinate IS		1.00	N/A	N/A	N/A	N/A		N/A	N/A	N/A



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Glyphosate

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N-acetyl glyphosate Group

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Glufosinate 3
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Glyphosate 2
Glyphosate 3
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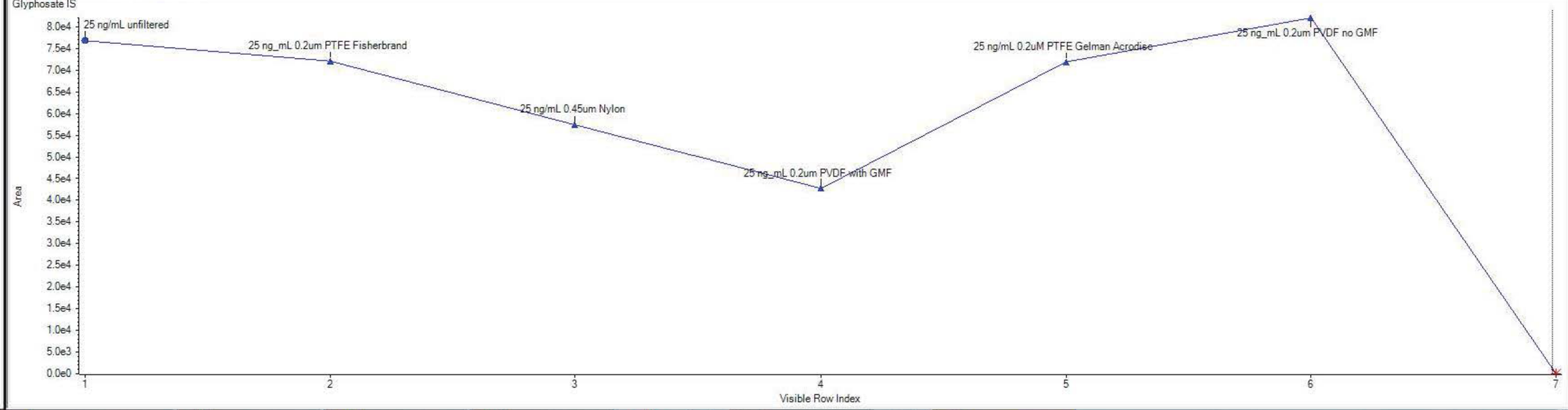
N-acetyl glyphosate 2
N-acetyl glyphosate 3

All Sample Types

Index	Sample Name	Sample Type	Dilution Factor	Component Name	Outlier Reasons	Actual Concentration	Area	Area Ratio	Height	Retention Time	Used	Calculated Concentration	Ion Ratio	Accuracy
25	25 ng/mL unfiltered	Standard	1.00	Glyphosate IS		1.00	76798	N/A	14074	4.39		N/A	0.7288	N/A
39	25 ng_mL 0.2um PTFE Fisherbrand	Quality Control	1.00	Glyphosate IS		1.00	72082	N/A	13427	4.40		N/A	0.7047	N/A
53	25 ng/mL 0.45um Nylon	Quality Control	1.00	Glyphosate IS		1.00	57374	N/A	11754	4.39		N/A	0.5762	N/A
67	25 ng_mL 0.2um PVDF with GMF	Quality Control	1.00	Glyphosate IS		1.00	42721	N/A	7931	4.42		N/A	0.4640	N/A
81	25 ng/mL 0.2uM PTFE Gelman Acrodisc	Quality Control	1.00	Glyphosate IS		1.00	71907	N/A	12929	4.40		N/A	0.7224	N/A
95	25 ng_mL 0.2um PVDF no GMF	Quality Control	1.00	Glyphosate IS		1.00	82047	N/A	16608	4.39		N/A	0.8458	N/A
109	H2O	Unknown	1.00	Glyphosate IS		1.00	N/A	N/A	N/A	N/A		N/A	N/A	N/A

Glyphosate IS



MultiQuant - [[MQ4] Results Table (20170202 Filters.qsession)]

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Glyphosate

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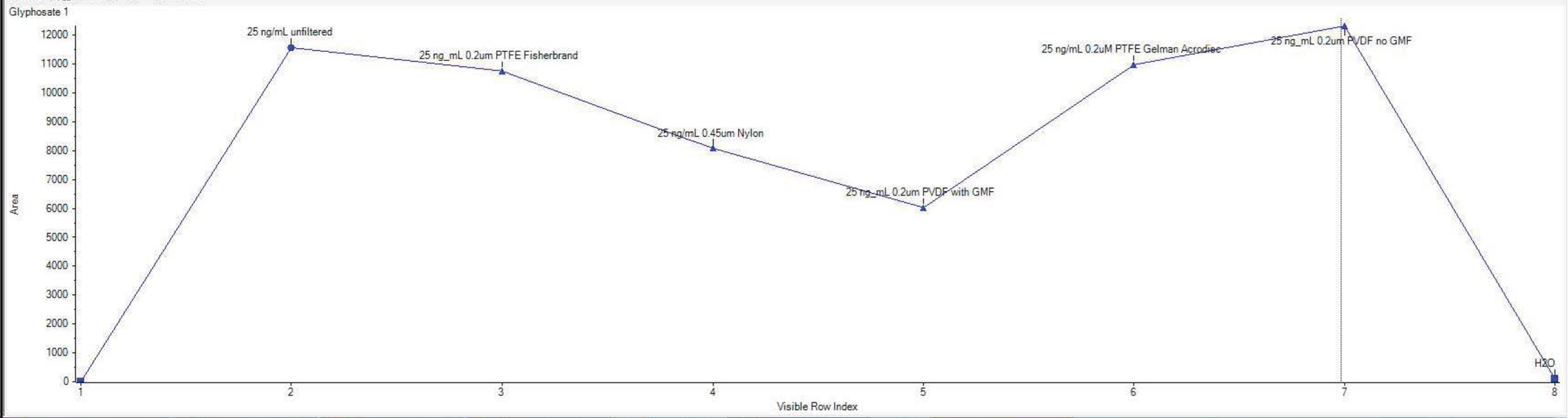
All Sample Types

Index	Sample Name	Sample Type	Dilution Factor	Component Name	Outlier Reasons	Actual Concentration	Area	Area Ratio	Height	Retention Time	Used	Calculated Concentration	Ion Ratio	Accuracy
8	Slvnt Blank	Unknown	1.00	Glyphosate 1	Ion Ratio	N/A	N/A	N/A	N/A	N/A	☑	N/A	N/A	N/A
22	25 ng/mL unfiltered	Standard	1.00	Glyphosate 1		25.00	11570	1.507e-1	2085	4.39	☑	25.0	1.0000	100.00
36	25 ng_mL 0.2um PTFE Fisherbrand	Quality Control	1.00	Glyphosate 1		25.00	10760	1.493e-1	1975	4.40	☑	24.8	1.0000	99.08
50	25 ng/mL 0.45um Nylon	Quality Control	1.00	Glyphosate 1		25.00	8081	1.409e-1	1647	4.39	☑	23.4	1.0000	93.49
64	25 ng_mL 0.2um PVDF with GMF	Quality Control	1.00	Glyphosate 1		25.00	6031	1.412e-1	1158	4.42	☑	23.4	1.0000	93.70
78	25 ng/mL 0.2uM PTFE Gelman Acrodisc	Quality Control	1.00	Glyphosate 1		25.00	10978	1.527e-1	1911	4.41	☑	25.3	1.0000	101.33
92	25 ng_mL 0.2um PVDF no GMF	Quality Control	1.00	Glyphosate 1		25.00	12319	1.501e-1	2393	4.40	☑	24.9	1.0000	99.66
106	H2O	Unknown	1.00	Glyphosate 1	Ion Ratio	N/A	98	N/A	51	4.41	☑	N/A	1.0000	N/A



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Standards

Sample Name	Sample Type	Dilution Factor	Component Name	Area	IS Area	Height	Retention Time	Actual Concentration	Calculated Concentration	Ion Ratio	Accuracy	Mass Info	Area Ratio
mL all 4	Standard	1.00	N-acetyl[210-63] 1	319	694650	147	5.31	5.00	4.8	1.0000	95.55	210.0 / 63.0	4.593e-4
mL all 4	Standard	1.00	N-acetyl[210-124] 2	5321	694650	511	5.37	5.00	17.8	16.6767	355.36	210.0 / 124.0	7.660e-3
mL all 4	Standard	1.00	N-acetyl[210-79] 3	44	694650	52	5.32	5.00	1.3	0.1384	25.88	210.0 / 79.0	6.359e-5
g/mL all 4	Standard	1.00	AMPA[110-63] 1	5955	114829	509	1.32	10.00	10.4	1.0000	103.74	110.0 / 63.0	5.186e-2
g/mL all 4	Standard	1.00	AMPA[110-79] 2	9119	114829	730	1.32	10.00	12.7	1.5314	126.95	110.0 / 79.0	7.941e-2
g/mL all 4	Standard	1.00	AMPA[110-81] 3	93	114829	86	1.37	10.00	16.9	0.0157	168.51	110.0 / 81.0	8.134e-4
g/mL all 4	Standard	1.00	AMPA[112-63] IS	114829	N/A	8315	1.33	1.00	N/A	1.0000	N/A	112.0 / 63.0	N/A
g/mL all 4	Standard	1.00	Glu[180-63] 1	20528	275947	2097	3.02	10.00	10.1	1.0000	101.22	180.0 / 63.0	7.439e-2
g/mL all 4	Standard	1.00	Glu[180-95] 2	32851	275947	3654	3.02	10.00	9.8	1.6003	97.55	180.0 / 95.0	1.190e-1
g/mL all 4	Standard	1.00	Glu[180-85] 3	34850	275947	3590	3.02	10.00	9.9	1.6977	99.01	180.0 / 85.0	1.263e-1
g/mL all 4	Standard	1.00	Glu[183-63] IS	275947	N/A	26823	3.00	1.00	N/A	2.4031	N/A	183.0 / 63.0	N/A
g/mL all 4	Standard	1.00	Gly[168-63] 1	73454	719436	16172	4.43	10.00	10.1	1.0000	100.64	168.0 / 63.0	1.021e-1
g/mL all 4	Standard	1.00	Gly[168-79] 2	59409	719436	12717	4.42	10.00	9.5	0.8088	94.79	168.0 / 79.0	8.258e-2
g/mL all 4	Standard	1.00	Gly[168-150] 3	327687	719436	68943	4.42	10.00	9.5	4.4611	95.24	168.0 / 150.0	4.555e-1
g/mL all 4	Standard	1.00	Gly[171-63] IS	719436	N/A	151921	4.42	1.00	N/A	6.2653	N/A	171.0 / 63.0	N/A
g/mL all 4	Standard	1.00	N-acetyl[210-63] 1	572	719436	241	5.34	10.00	8.3	1.0000	82.64	210.0 / 63.0	7.946e-4
g/mL all 4	Standard	1.00	N-acetyl[210-124] 2	210	719436	87	5.25	10.00	0.7	0.3681	6.78	210.0 / 124.0	2.925e-4
g/mL all 4	Standard	1.00	N-acetyl[210-79] 3	163	719436	123	5.31	10.00	4.6	0.2846	46.02	210.0 / 79.0	2.261e-4
g/mL all 4	Standard	1.00	AMPA[110-63] 1	33539	109430	2589	1.33	50.00	61.3	1.0000	122.63	110.0 / 63.0	3.065e-1
g/mL all 4	Standard	1.00	AMPA[110-79] 2	41783	109430	3631	1.32	50.00	61.0	1.2458	122.08	110.0 / 79.0	3.818e-1

Apply

Manual Integration

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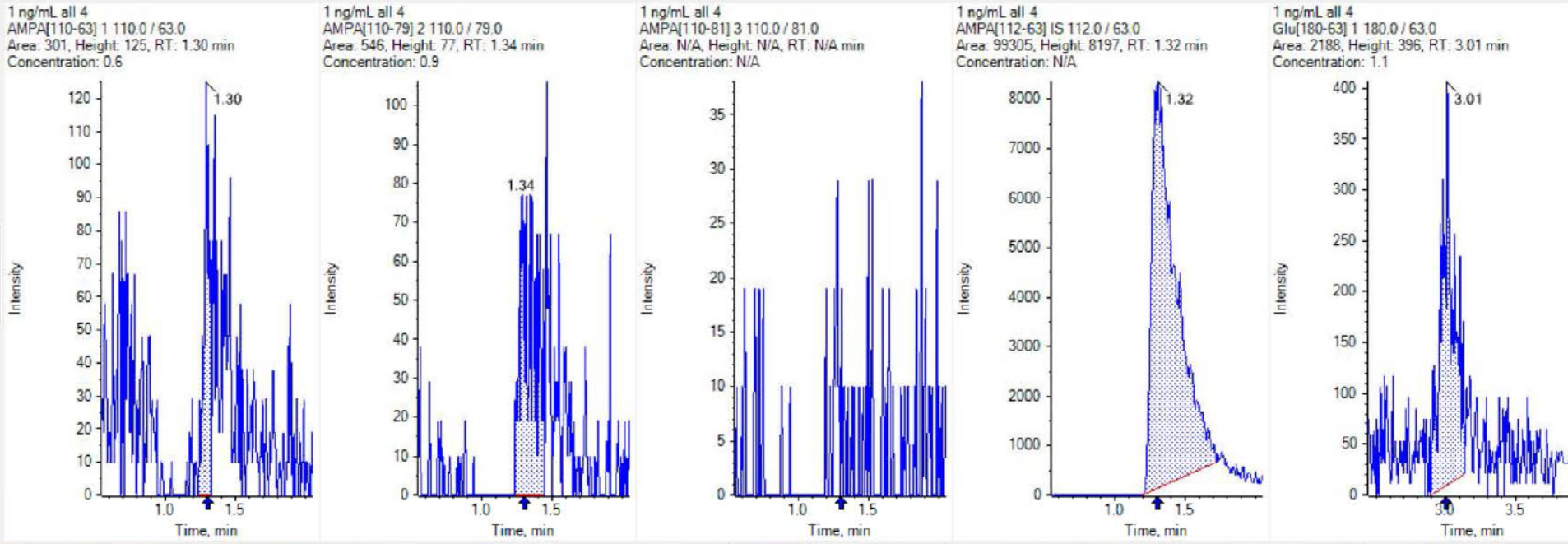
4 points



Multi-Laboratory Collaborative Study Validation Plan: Quantification of Sulfites in Foods and Drink Samples

Validation Study Point of Contact (POC):

Katherine Robbins
FDA/CFSAN/ORS/DAC/MDB
240-402-1835
katherine.robbins@fda.hhs.gov

Method POC:

Katherine Robbins
FDA/CFSAN/ORS/DAC/MDB
240-402-1835
katherine.robbins@fda.hhs.gov

Scope:
This validation plan outlines a collaborative study that follows a level four validation as outlined in the FDA's *Guidelines for the Validation of Chemical Methods for the FDA Foods Program.* This validation level is equivalent to a full AOAC Collaborative study. This validation level was chosen since it is expected that the use of this methods will be widespread, long term and of high public visibility. A level two single laboratory validation was completed and published in *Journal of Agricultural and Food Chemistry* in the spring of 2015.

This collaborative study will test the performance of an LC-MS/MS method for the quantitation of sulfites in a wide range of food products. Sulfites are a family of food preservative which can cause severe allergic-like reactions in sensitive individuals. As a result, the U.S. FDA requires that sulfite be listed on the label of any food product containing more than 10 ppm sulfite (measured as sulfur dioxide). [1, 2]

Method:
The multilaboratory validation study will test the performance of a method that was published by Robbins *et al*. in the *Journal of Agricultural and Food Chemistry* in 2015. This method uses electrospray ionization (ESI) liquid chromatography–tandem mass spectrometry (LC-MS/MS) for the determination of sulfite in various food matrices. The matrix is extracted with a buffered formaldehyde solution, converting free and reversibly bound sulfite to the stable formaldehyde adduct, hydroxymethylsulfonate (HMS). Extracts are prepared for injection using a C18 SPE cartridge to remove any lipophilic compounds. HMS is then separated from other matrix components using hydrophilic interaction chromatography (HILIC) and detected using multiple reaction monitoring (MRM). Slight modifications in the sample preparation steps were made for liquids and dried vegetables.

Potential Participating Labs:

- CFSAN
- FCC
- Denver (ORA)
- New York (ORA)
- Irvine (ORA)
- San Francisco (ORA-no QQQ)
- Florida Department of Agriculture (FERN)
- New York Department of Agriculture (FERN)
- Minnesota Department of Agriculture (FERN)

Study Design:
The validation study will consist of two parts starting with training and proficiency samples followed by the actual collaborative samples. In the initial stage of the study, laboratories will be provided with an excess of a standard solution to be used to ensure the LC-MS system is suitable for the analysis. Two spiked samples (one liquid and one solid) will be analyzed in duplicate to prove the analyst has proficiency with the method. After this preliminary evaluation is successfully completed, the collaborative study samples will be sent. Four different representative matrices will be spiked with varying concentrations of Na_2SO_3. Table 1 outlines these samples as well as if any modifications will be required for that sample. Three commercially sulfited samples will also be analyzed. All samples will be analyzed as blind duplicates.

Participating laboratories will receive the S^{34}-sodium sulfite internal standard stock solution (IS), a stock solution of sodium sulfite, the analytical column, and the SPE cartridges to be used throughout both phases of the experiment. The spiked samples will include a matrix blank with sodium sulfite added at three concentrations to best represent the concentrations usually encountered in those matrices. The liquid sample, white grape juice, will be spiked at 0x, 1/2x, 1x, and 2x the regulatory labeling threshold level of 10 ppm SO_2.

Instructions for Participating Laboratories:

1. Once the training samples have arrived, begin familiarizing yourself with the procedures and instrumentation of the method. The initial training solution will be provided in excess to allow for multiple analyses.
2. Follow the method procedures carefully. Ensure that good laboratory practices are followed. This is a study of the method, not of the laboratory. The method must be followed as closely as possible and any deviations from the method must be recorded and reported to the study point of contact.

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Recommended Analysis Schedule:

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Reporting:
All data reports are entered into the Sulfite Data Template Excel workbook. The completed excel workbook should be sent to the Validation Study POC. In addition, copies of all chromatograms should be sent to the POC as well.

References:
1. U.S. Food and Drug Administration, Fed. Regist. 1986, 51, 25012-25020.
2. U.S. Food and Drug Administration, Fed. Regist. 1986, 51, 25021-25026.

Table 1: Multi laboratory Validation Samples

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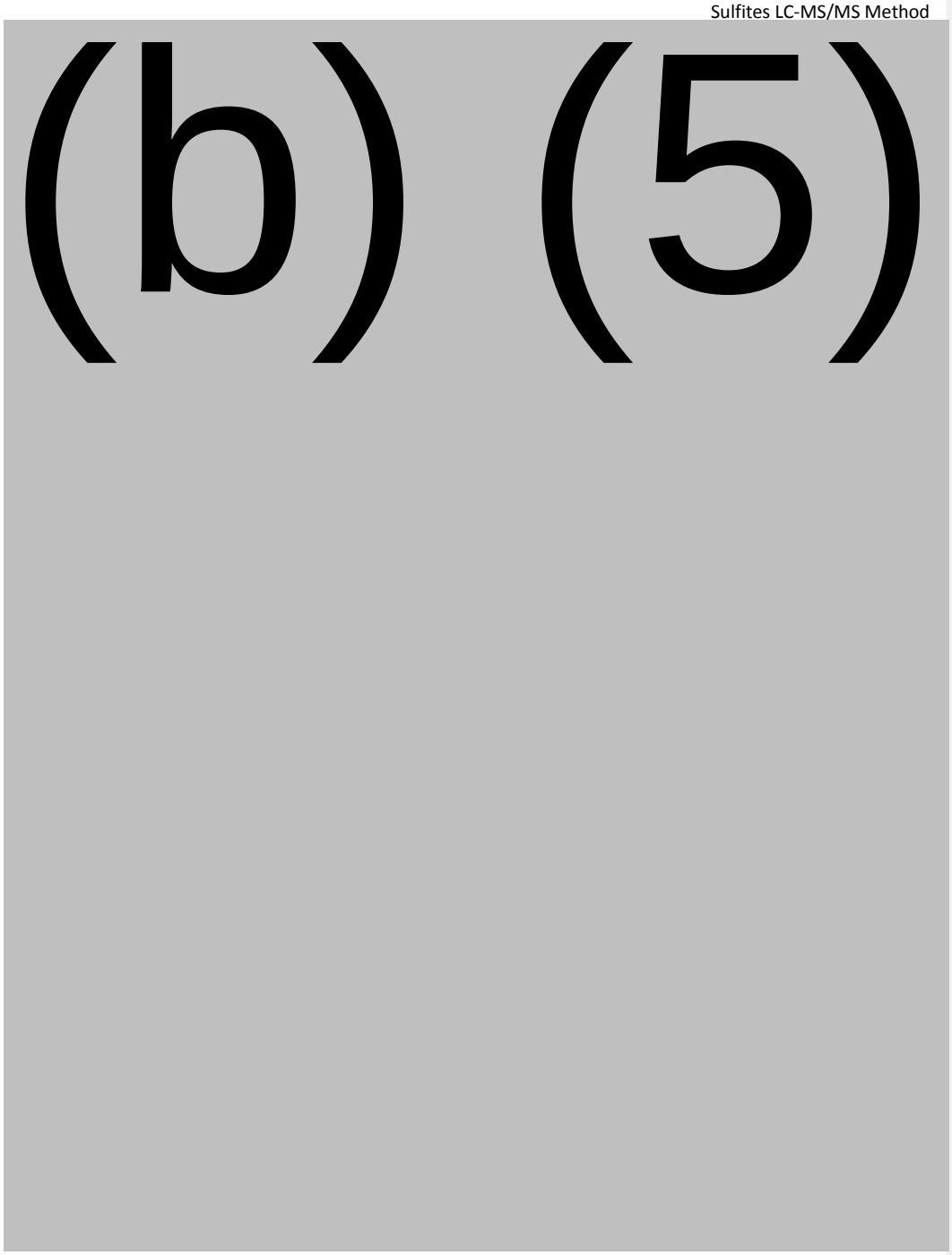
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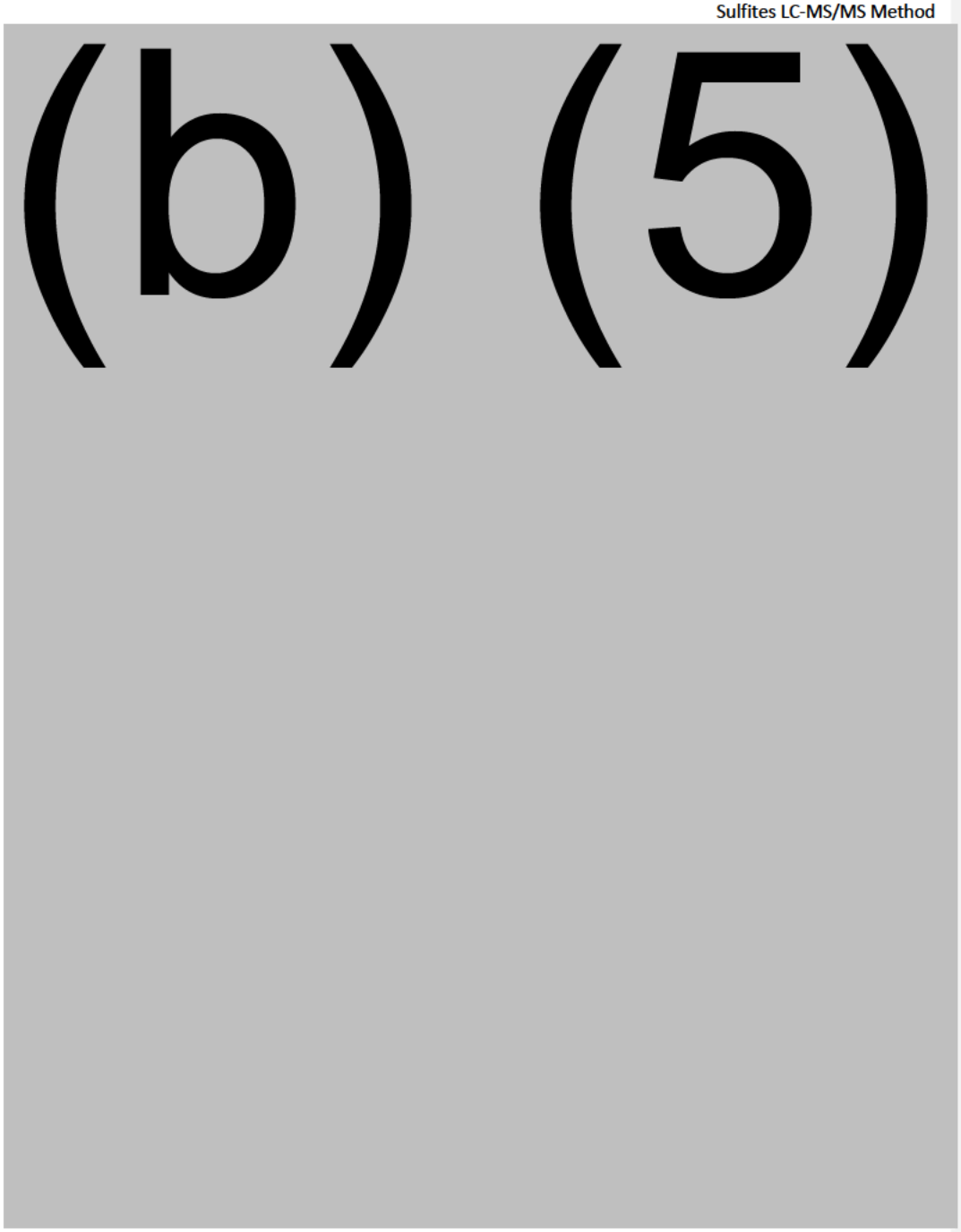
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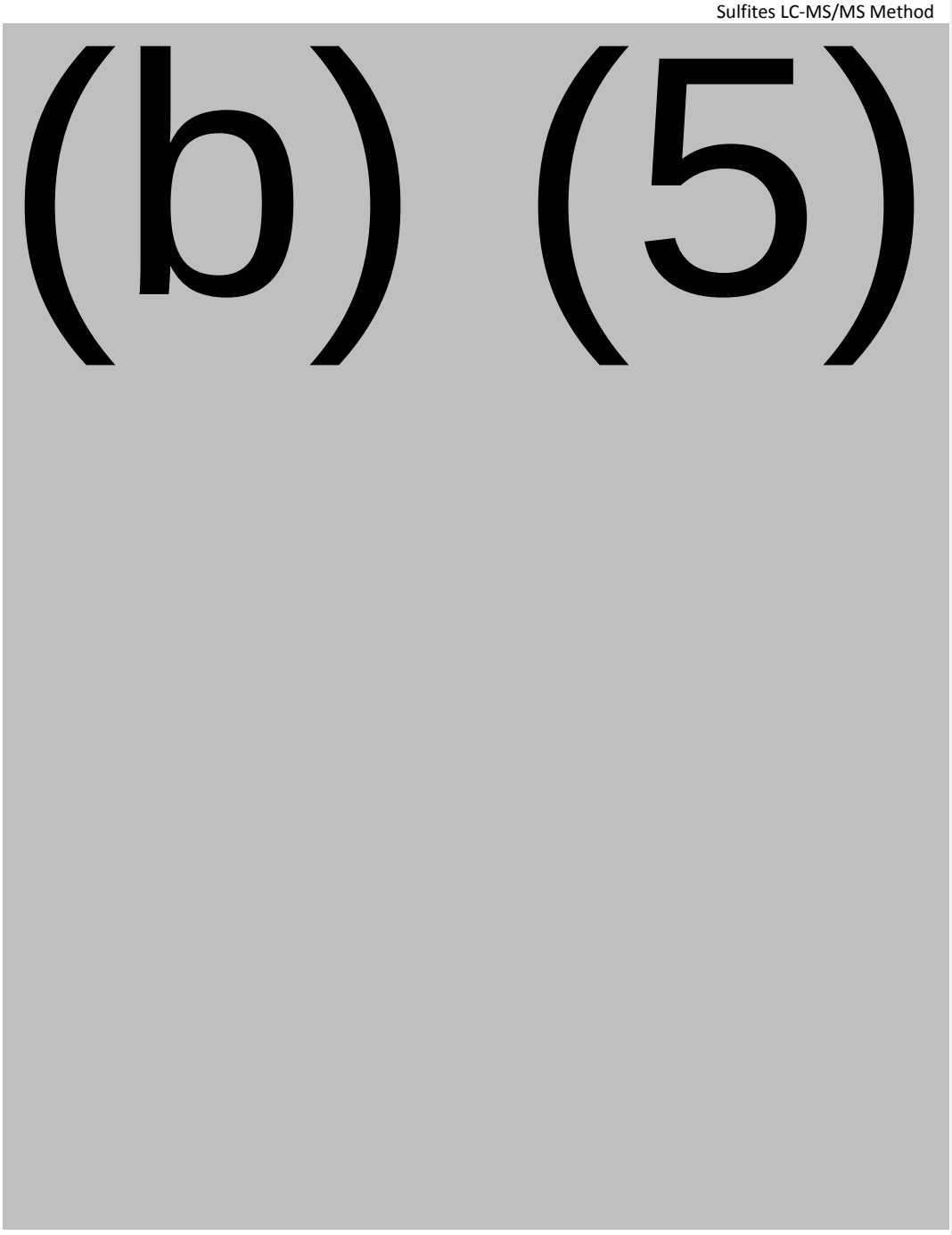
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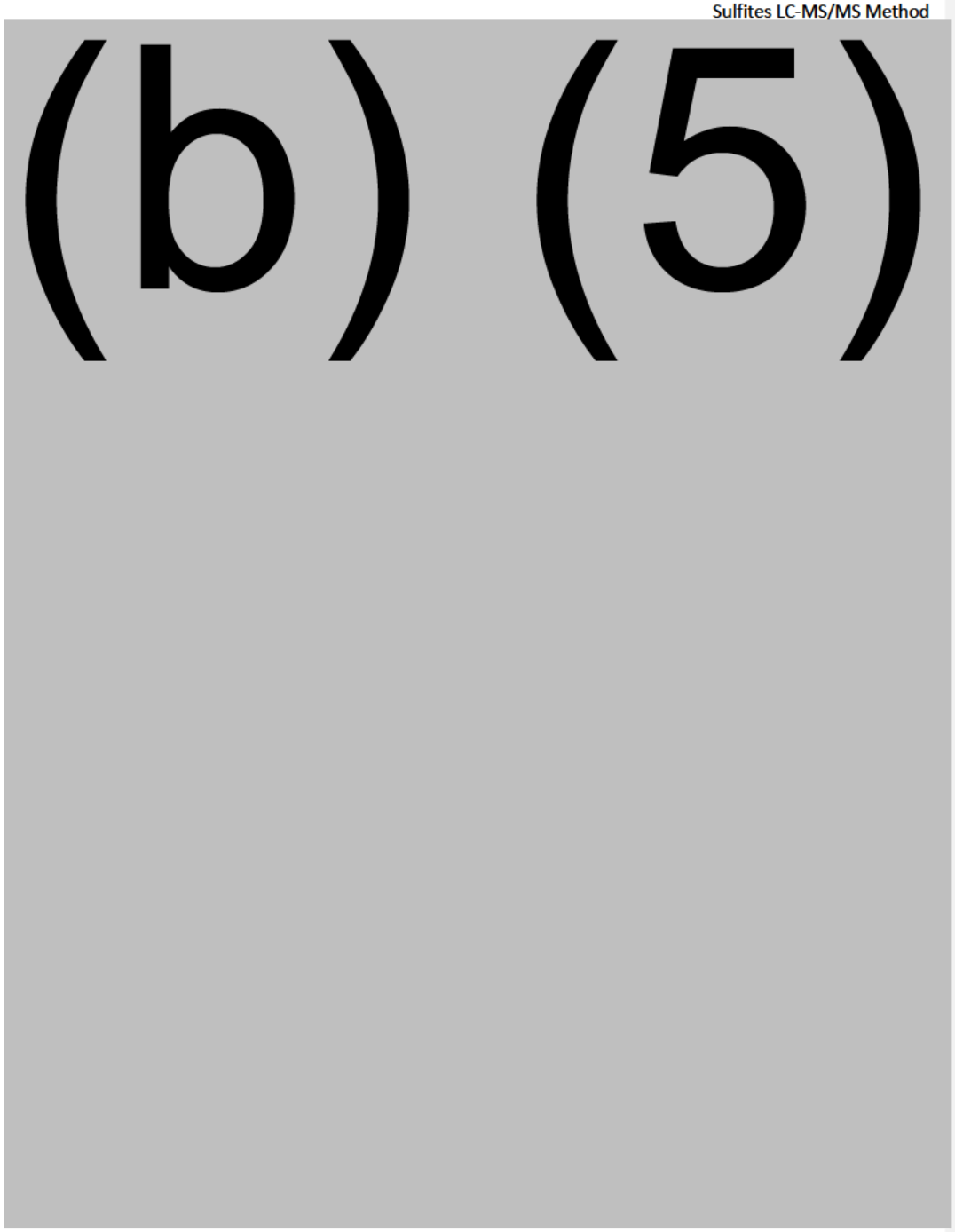
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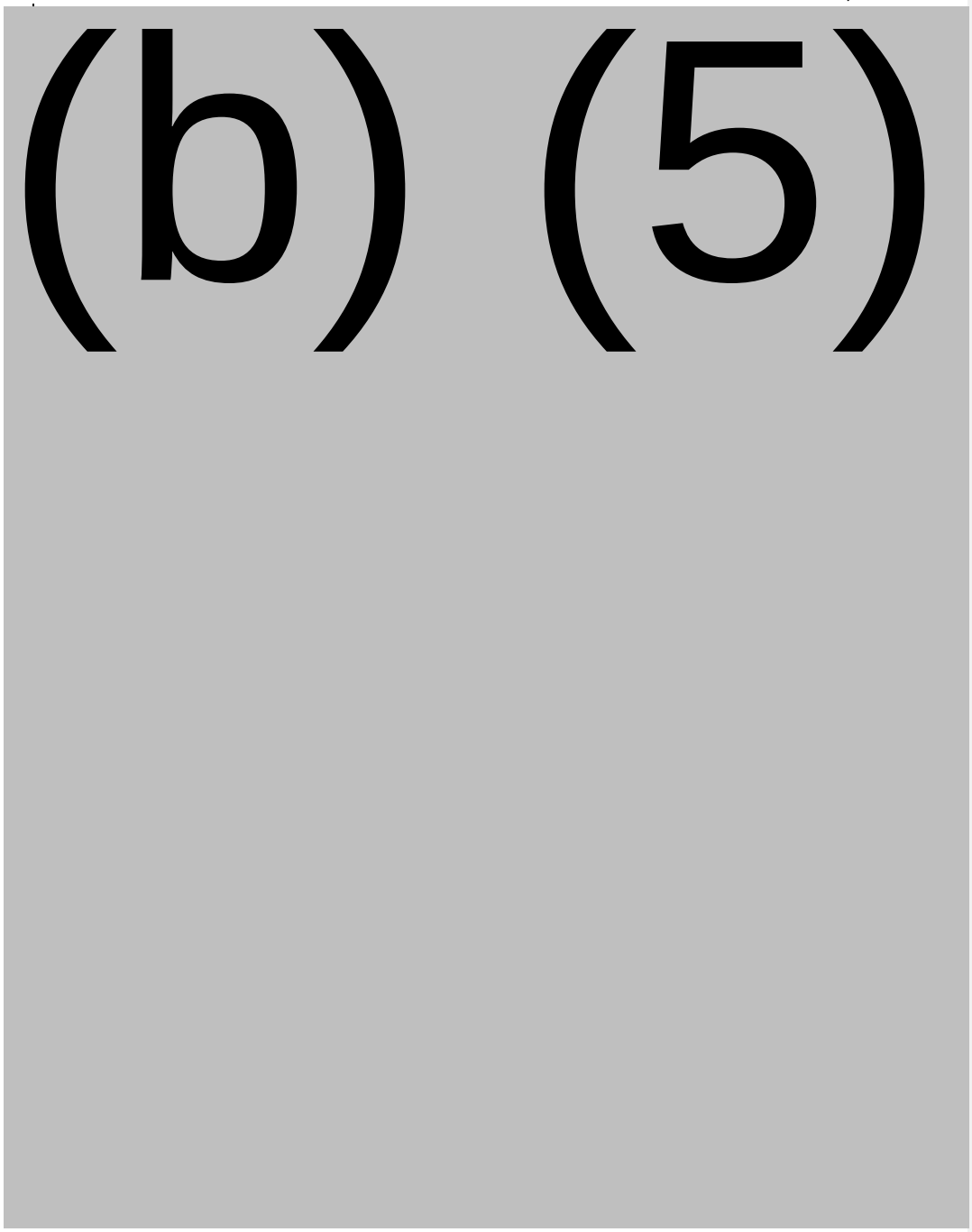
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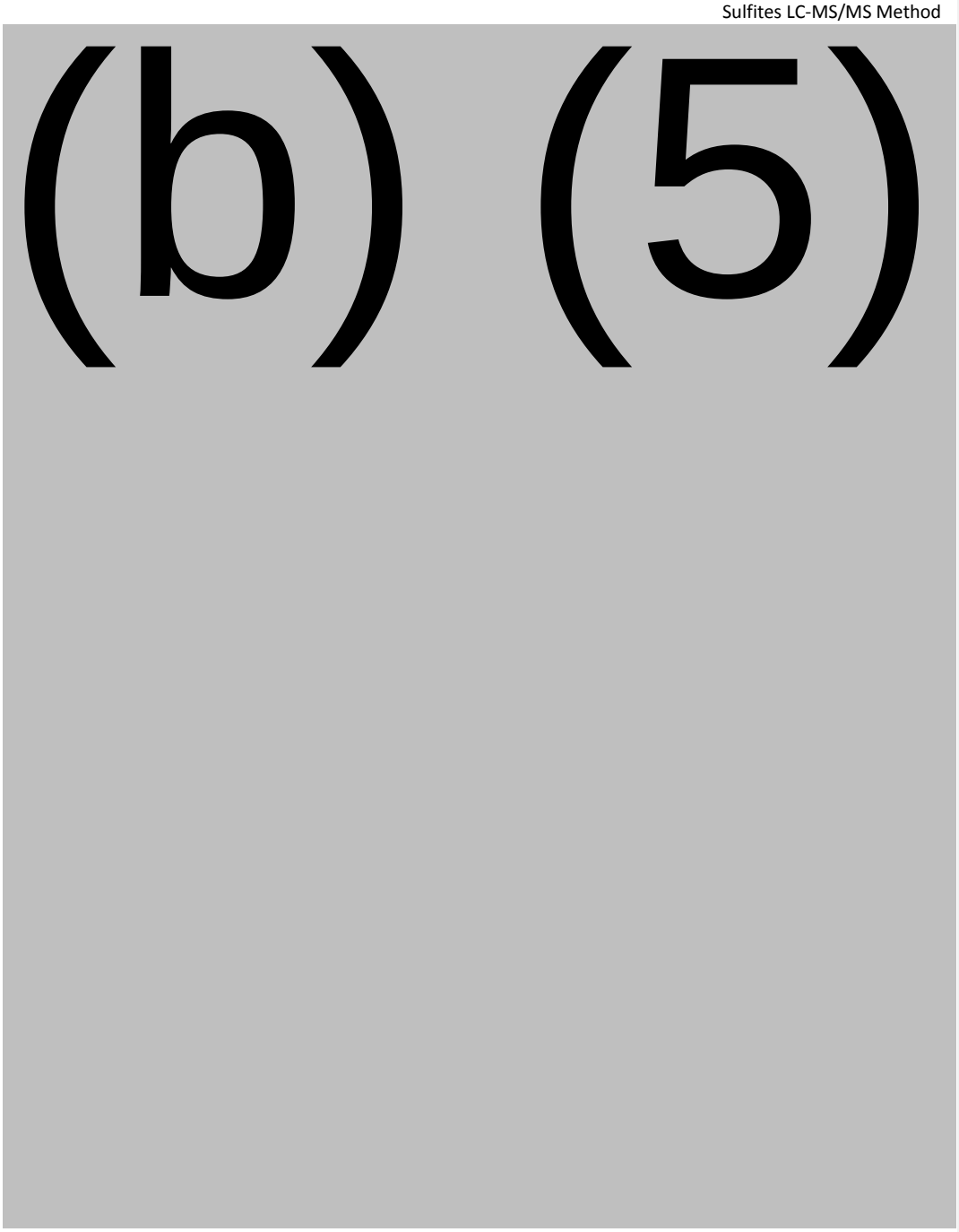
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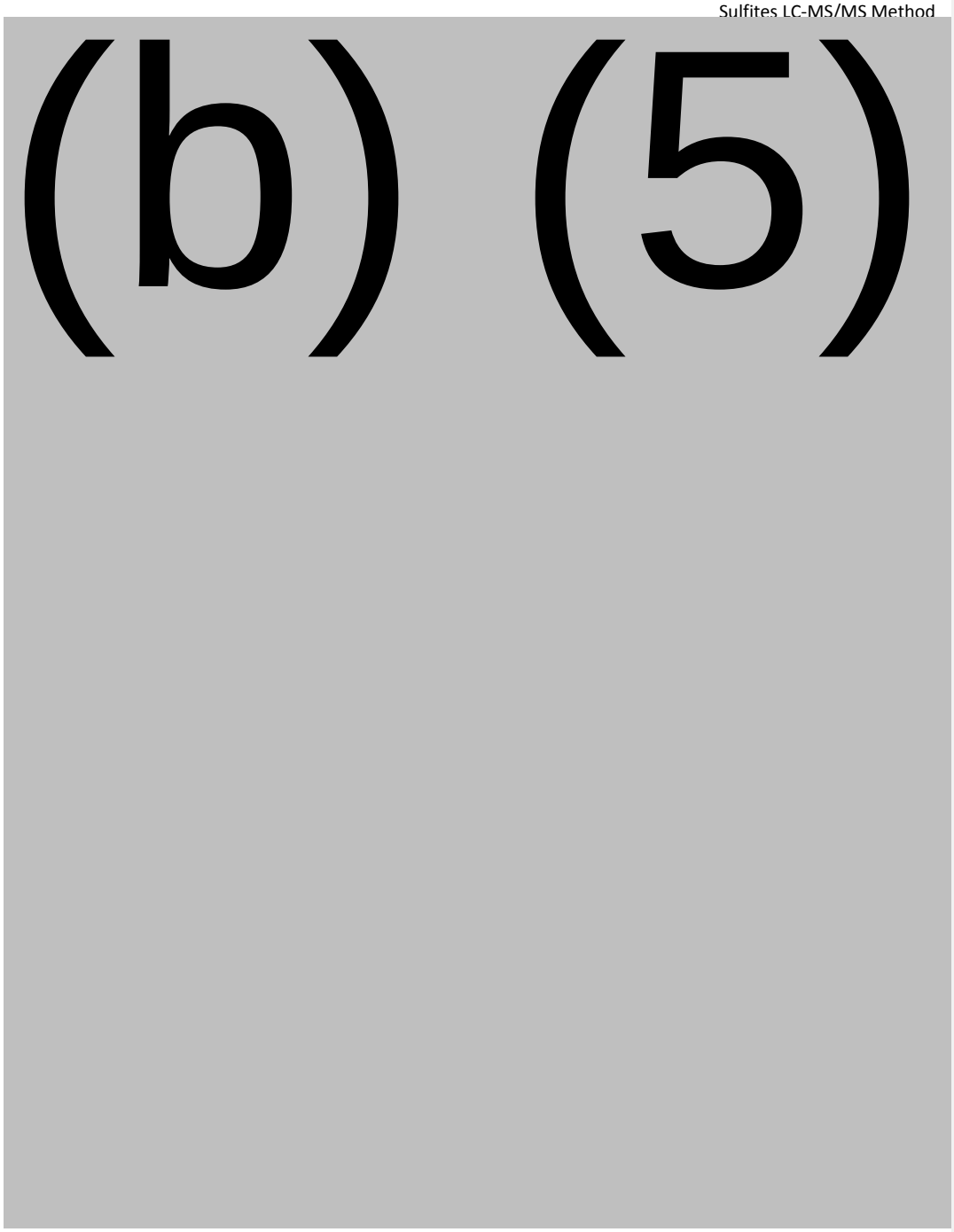
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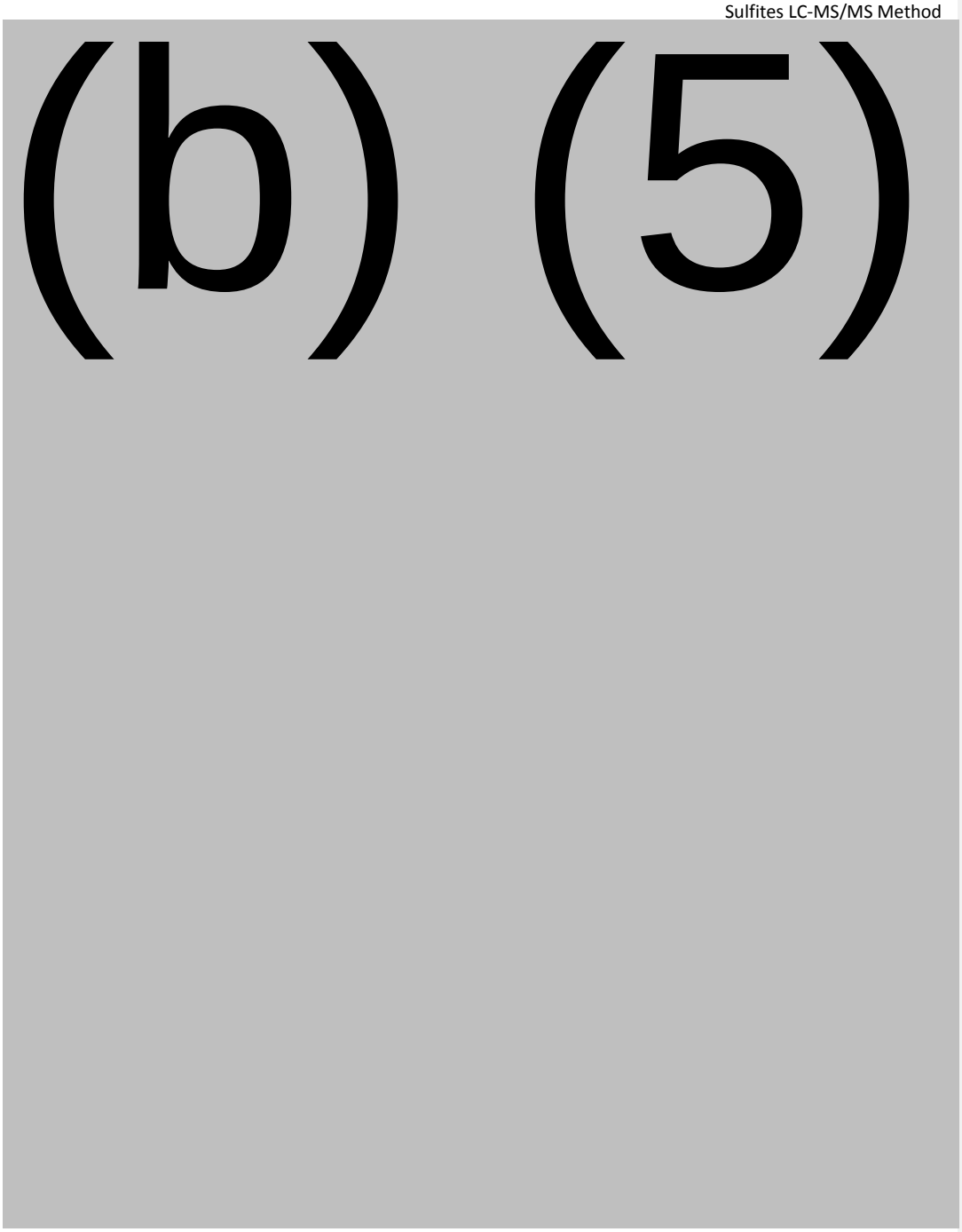
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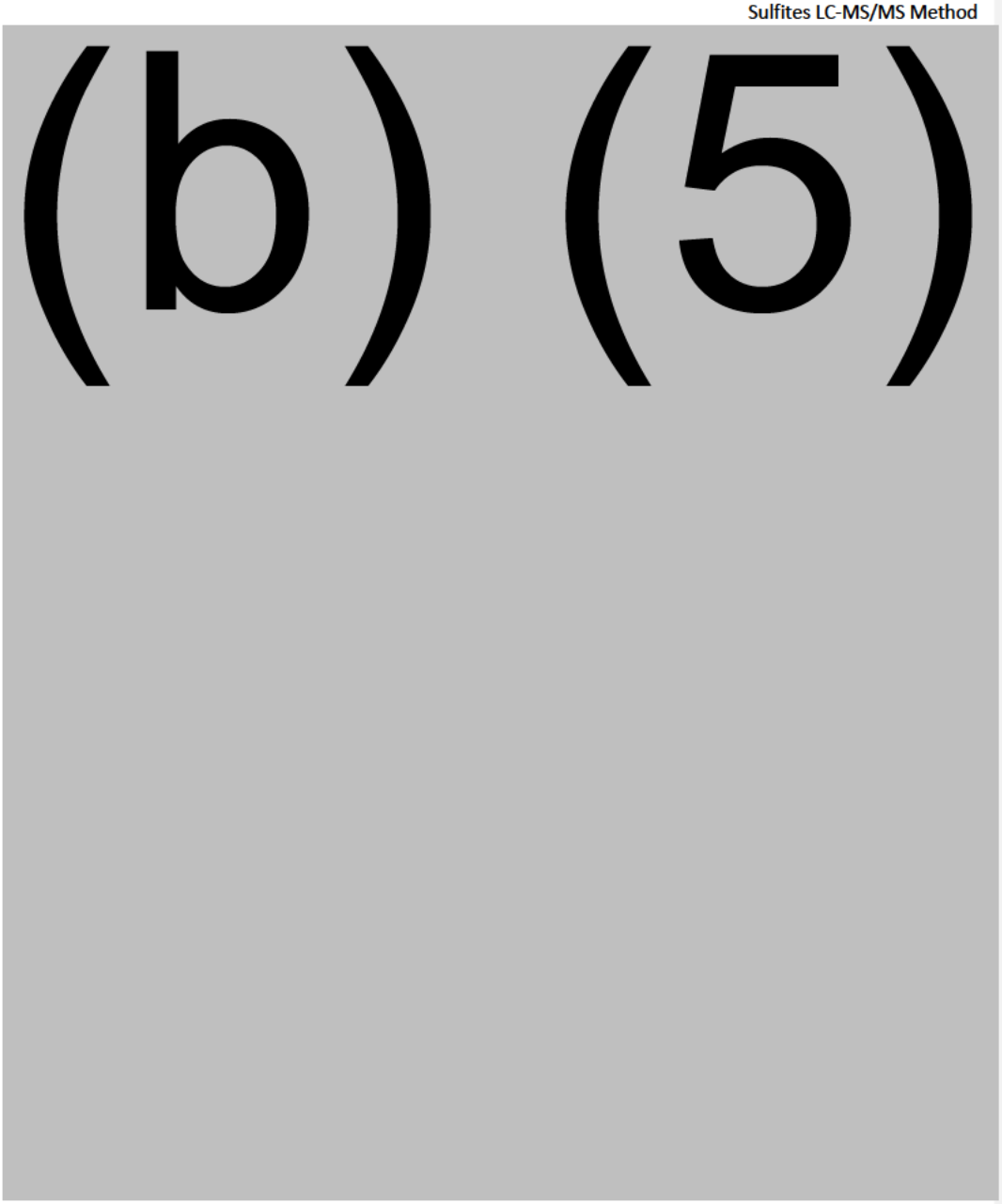
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Matrices: Corn (dry)
Carrot (wet)
Avocado (fat)

Analyses: (per matrix)

Fortification Study

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

Incurred Residues

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

Standards:

Calibration Standards in Solvent

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

Matrix Calibration Standards

Std Conc (ng/ml)	Spk Std[1] Conc (µg/ml)	Spk Std Volume Added (µl)	Dilution[3] Volume (ml)
corn (2 g sample)			
10	1	40	4
50	5	40	4
100	5	80	4
carrot/avocado (5 g sample)			
10	1	100	10
50	5	100	10
100	50	20	10

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

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

[3] *Dilute with control sample matrix*

Fortification:

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

LCMS Transitions: AMPA[110-63] 1
AMPA[110-79] 2
AMPA[110-81] 3
Glu[180-63] 1
Glu[180-95] 2
Glu[180-85] 3
Glu[183-63] IS
Gly[168-63] 1
Gly[168-79] 2
Gly[168-150] 3
Gly[171-63] IS
N-acetyl[210-63] 1
N-acetyl[210-124] 2
N-acetyl[210-79] 3

LCMS Calibration: Single level calibration for each spike level
Internal standard calibration: Glyphosate & Glufosinate
External standard calibration: AMPA & N-acetyl-glyphosate

Inj Sequence: Group by spike level

Description	Sample Name	Sample Type	Concentration

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

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

Calc concentration