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THE LEADER IN ENVIRONMENTAL TESTING

Fast Analysis of Chlorinated Phenoxy Herbicides in Environmental Samples by Negative ESI LC-MS/MS

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OUTLINE

- Current Test Method
- Why New Method (LC-MS/MS)?
- Instrument Parameters and Conditions
- Results and Discussions
- Method Validation
- Summary
- Acknowledgement

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GC-ECD - Herbicides

- EPA 8151A
  - ~ Rev 1, December 1996
- EPA 8150B
  - ~ Rev 2, September 1994
- EPA 615, 1658
  - ~ Herbicides in waste water

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EPA 8151 Limits

- EPA 8151 Detection limits*

Analyte	Water		Solid	
	MDL (ug/L)	RL (ug/L)	MDL (ug/Kg)	RL (ug/Kg)
Dalapon	3.98	12	38.5	40
2,4-D	2.07	8	22	80
Dicamba	0.29	2	3.93	40
MCPA	132	400	3071	8000
MCPP	142	400	4796	8000
Dichlorprop	1.70	5	23.4	80
2,4,5-T	0.17	1	7.93	20
2,4-DB	2.62	8	43.8	80
2,4,5-TP (Silvex)	0.15	1	3.3	20
Dinoseb	0.63	2	13.6	25

*Data from TALS – St. Louis Lab.

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Why a New Method?

- Reporting Limits not low enough by method 8151A
  - ~ Lower regulatory limits, especially for ground water, especially poor responders: MCPA, MCPP
- Method 8151 non-specific target Identification
  - ~ Relies on RT and secondary column confirmation
- GC-ECD method more subject to Interference
  - ~ Peaks with similar RT
  - ~ Complex environmental sample matrices
- Method 8151A requires 1 Liter sample volume
  - ~ Difficult in arid climates, drought conditions
- TAT 5- 10 days on average
- Complex method preparation; poor recoveries

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LC-MS/MS Method

- An alternative to method 8151
  - ~ Based on EPA 8321 method (TS-LC/MS)
  - ~ Covers 8151 target list
  - ~ Employs a more specific detection mechanism
  - ~ Safer and simple sample preparation
  - ~ Requires a much smaller sample size
  - ~ Allows for faster turnaround times
  - ~ No sacrificing sensitivity

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PROS and CONS

- **EPA 8151 (GC-ECD)**

  CONS
  - ~ Derivatization is needed
  - ~ Excessive solvent use
  - ~ Large sample aliquot for prep (1L)
  - ~ Time consuming prep (1-2 days)
  - ~ TAT – 5 or more days
  - ~ Less selectivity - Second column confirmation is needed
  - ~ Matrix interference on GC
  - ~ Less sensitivity

  PROS
  - ~ Well-known method
  - ~ Ready for GC data validation
  - ~ Less startup cost (GC-ECD)

- **EPA 8321 (LC-MS/MS)**

  PROS
  - ~ Direct aqueous extract injection
  - ~ Solvent: methanol, acetonitrile
  - ~ Small sample aliquot for prep (10 mL)
  - ~ Prep time: 2 hours
  - ~ TAT – 24 hours is achievable
  - ~ High selectivity with MRM mode - No confirmation run is needed
  - ~ Matrix interference minimized
  - ~ Lower detection limits (sub-ppb level)

  CONS
  - ~ New method (comparability)
  - ~ Training may be needed for LC-MS/MS data validation
  - ~ High startup cost (LC-MS/MS)

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Sample Preparation

- Procedure
  - ~ Sample size: 10mL of water, 2 grams of soil
  - ~ Solvent use: Acetonitrile and methanol
  - ~ Hydrolysis: adjust pH ≥ 12
  - ~ Shake for 1 hour
  - ~ Acidification: adjust pH 2-3
- No derivatization
  - ~ Does not utilize diazomethane for derivatization
  - ~ Reducing staff exposure to hazardous chemicals (ether …)
  - ~ Eliminating the cost of transport and disposal of a potentially explosive forming diazo compound.
- Added hydrolysis reaction from direct injection prep
  - ~ Hydrolysis converts ester forms into their corresponding carboxylic acids.
  - ~ Regardless of original molecular forms in the native sample the herbicide concentration will be accounted for in the final result.

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LC-MS/MS System

- Waters Acquity UPLC
- Waters Xevo TQ MS (MS/MS system)

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LC Considerations

- Short run time (12 minutes cycle time)
  - ~ Lower resolution
  - ~ Some peaks may not be separated
- LC column
  - ~ Good for separating polar compounds
  - ~ Stable for use in acid mobile phase (pH 2-3)
  - ~ Acquity UPLC BEH C18, 1.7um x 2.1mm x 100mm
- Mobile phase (0.1% acid)
  - ~ Acid selection (volatile)
    - Formic acid (HCOOH)
    - Acetic acid (CH3COOH)
    - Phosphoric acid (H3PO4)

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MS/MS Parameters

- Uses ESI Negative Ion
- MRM (Multiple Reaction Mornitoring)

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MRM and Scan Conditions

Analyte	MRM (m/z)	Cone Volt (V)	Collision Energy (eV)
Dalapon	141 > 97	15	10
2,4-Dichlorophenyl acetic acid (DCAA), surrogate	249 > 159	15	12
2,4-D-d3, surrogate	222 > 164	25	13
2,4-D	219 > 161	25	13
Dicamba	219 > 175	10	7
MCPA	199 > 141	18	15
MCPP	213 > 141	20	14
Dichlorprop	233 > 161	25	14
2,4,5-T	253 >195	20	12
2,4-DB	247 > 161	15	12
2,4,5-TP (Silvex)	267 > 195	17	15
Dinoseb	239 > 193	35	25
2,4-Dichlorophenoxyacetic acid (13C6), IS	225 > 167	25	13
2,4,5-Trichlorophenoxyacetic acid (13C6), IS	259 > 201	20	12

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Results and Discussions

- Initial calibration
- Sensitivity and selectivity
- Method stability test
- pH effect
- Validation / PT results

Calibration and Sensitivity

- Initial Calibration
  - ~ Good linearity over the calibration range: 0.2 ppb to 50 ppb
- LOD and LOQ result show excellent sensitivity especially for soil

Analyte Name	Calibration %RSD/r2	Water		Solid	
		LOD (ug/L)	LOQ (ug/L)	LOD (ug/Kg)	LOQ (ug/Kg)
Dalapon	16	0.82	2 (12)*	1.9	10 (40)*
2,4-D	14	0.4	2 (8)	1.5	5 (80)
Dicamba	10	0.57	2 (2)	1.4	5 (40)
MCPA	10	0.69	5 (400)	2.3	10 (8000)
MCPP	16	0.75	5 (400)	2.9	10 (8000)
Dichlorprop	0.996	0.46	2 (5)	2.3	5 (80)
2,4,5-T	7.7	0.57	1 (1)	2.9	5 (20)
2,4-DB	9.9	0.17	2 (8)	2.5	5 (80)
2,4,5-TP (Silvex)	10	0.5	1 (1)	1.6	5 (20)
Dinoseb	20	0.15	2 (2)	0.8	5 (25)

* (GC 8151 reporting limit)

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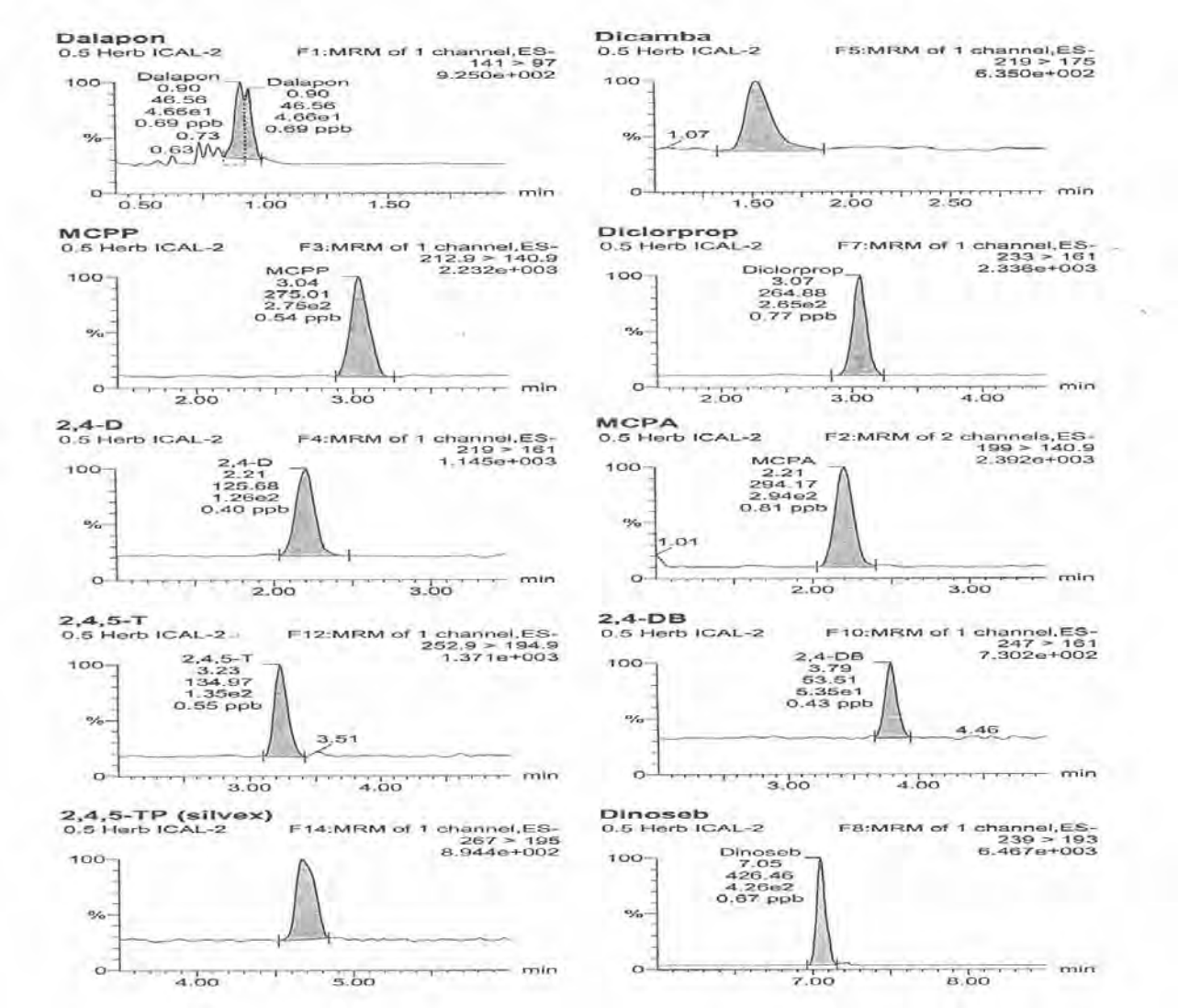
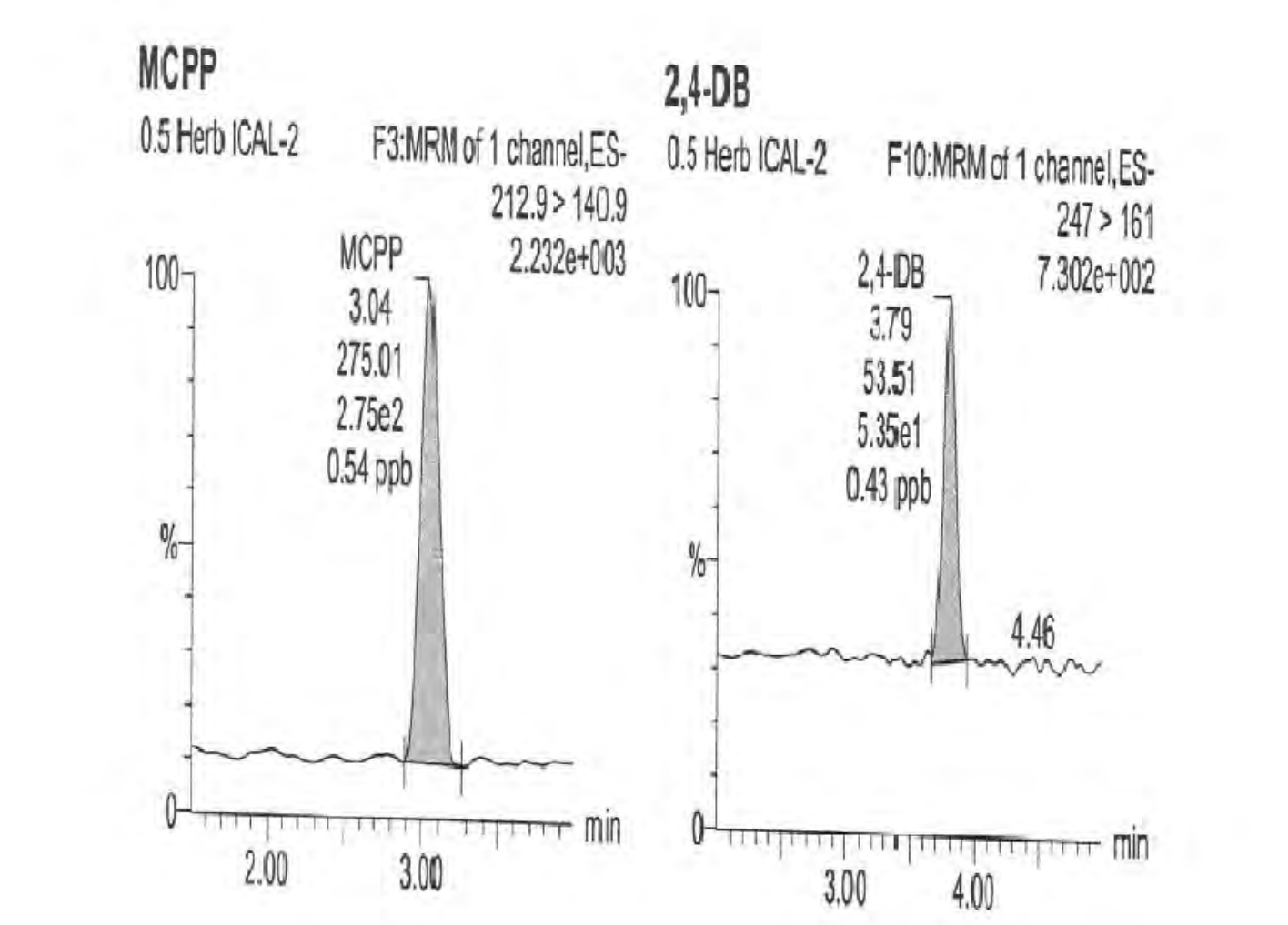
MRM Spectrum



Note:
Conc=0.5 ppb
pH = 3

From the spectrum
- Nice peak shape
- Good S/N ratio
- RT overlaps
- Free interference within the window

MRM Spectrum



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Method Stability

- A key factor for commercial testing laboratories
- Test Matrix
  - ~ HPLC water and muffled silica sand
  - ~ Real world aqueous and soil samples
- Tests performed
  - ~ Continuous Calibration Verification tests
  - ~ Internal standard recovery test

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Method Stability - CCVs

- Continuous CCV Recoveries in 48 hours

Analyte	CCV (%Recovery)					CCV S. Dev
	1	2	3	4	5	
Dalapon	98.5	94.8	97.6	100.8	113.5	7.3
2,4-D	76.5	101.8	81	93.1	107.7	13.3
Dicamba	86.1	99.4	91	107.7	102.3	8.7
MCPA	88.6	105.2	96.2	102.7	114.7	9.8
MCPP	91.3	105.1	97.9	95.2	82.6	8.3
Dichlorprop	86.5	98.4	105.7	97.4	110.3	9.1
2,4,5-T	95	109.9	83.7	71.3	108.2	16.4
2,4-DB	115.7	122.4	107.1	106.4	113.4	6.6
2,4,5-TP (Silvex)	88.1	106.9	93.4	90.2	97.1	7.4
Dinoseb	103.3	97.5	87.6	82.1	89.9	8.4
DCAA, surrogate	122.9	103.7	108.4	94.9	112.4	10.4

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Method Stability – Internal STD

Internal Standard	% Recovery										Avg %
	1	2	3	4	5	6	7	8	9	10	
2,4-D (13C6)*	121.3	116.5	114.4	108.5	118.3	125.7	117.6	107.7	124.3	107	**116**
2,4,5-T (13C6)*	115.5	111.7	112	105.1	123.4	118.8	113.6	107.2	100.8	107.8	**111**
2,4-D (13C6)**	116.2	88.7	91.6	92.8	95.5	97.8	110.9	86.9	99.1	84.3	**96**
2,4,5-T (13C6)**	112.8	95.7	97.9	89.8	84.4	95.7	96.3	100.4	103.3	93.7	**97**

10 Continuous injections from *batch on 6/14/2012.

**batch on 3/14/2014.

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Method Stability

- CCV recoveries are generally within 80-120%
- Internal standard recoveries are within 70-130%

With over one year of testing, the method stability overall remains adequate and stays stable for days in between initial calibrations.

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Importance of pH

- Sample prep
  - ~ Hydrolysis pH > 12
  - ~ Acidification pH = 2-3
- Instrument analysis
  - ~ Mobile phase pH = Extract pH
  - ~ If pH off the range

Consistent pH is the key to achieve the best results!

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Method Validation Protocol

- The experimental design will be used to establish the validity of the analytical method
  - Reagent, solvents
  - Sample, standard, solution preparation
  - Instrument system condition and suitability
  - Software and calculations
- Results are proven meeting the goal of the method design in terms of prep, linearity and range, sensitivity, selectivity, and repeatability
  - Prep: catch the total amount of each herbicide in the target list regardless their initial molecular forms in the sample
- Accuracy has to agree with a reference value
  - comparison to the results of the proposed analytical procedure: parallel testing vs 8151
  - comparison to the results of reference materials: PT performance

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Method Validation

- Parallel testing 8151 vs 8321 (acid STD)
  - ~ Same matrix, standards, spike amounts
  - ~ Follow standard procedures of 8151/8321
  - ~ Results are comparable
- Prep validation
  - ~ Mixed forms (acid + esters) STD
  - ~ Acid form STD
- PT results
  - ~ 8151 vs 8321, a comparison
  - ~ More 8321PTs for both water and soil

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Method Validation - Prep

- Herbicide (mixed forms STD) Recovery with/without hydrolysis

Target	Compound Name	STD Conc (ppm)	Spiked ug/L	Amt measured without hydrolysis ug/L	%Rec without hydrolysis	Amt measured with hydrolysis ug/L	%Rec with hydrolysis (total)
2,4 - D	2,4 – D (acid)	100	5	4.68	93.6 (acid only)	acid	
	2,4-D 2-ethylhexyl ester	100	5	ester	ester	acid	
	2,4-D butoxyethanol ester	100	5	ester	ester	acid	
	Total		15	4.68	31	11.3	75.3
2,4,5 - T	2,4,5 – T (acid)	100	5	4.95	99 (acid only)	acid	
	2,4,5-T butyl ester	100	5	ester	ester	acid	
	2,4,5-T butoxyethanol ester	100	5	ester	ester	acid	
	Total		15	4.95	33	11.1	74

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Method Validation - Prep

- Herbicides (acid form STD) recovery with/without hydrolysis

Target	STD Conc (ppm)	Spiked ug/L	Amt measured without hydrolysis ug/L	%Rec without hydrolysis	Amt measured with hydrolysis ug/L	%Rec with hydrolysis
2,4-DB	100	5	4.18	**83.6**	4.20	**84**
Dalapon	100	5	4.10	**82**	5.16	**103.2**
Dicamba	100	5	4.86	**97.2**	5.43	**108.8**
Dichlorprop	100	5	4.39	**87.8**	5.18	**103.6**
Dinoseb	100	5	4.97	**99.4**	4.50	**90**
MCPA	100	5	3.98	**79.6**	4.16	**83.2**
MCPP	100	5	5.24	**104.8**	5.87	**117.4**
2,4,5-TP	100	5	4.95	**99**	5.13	**102.6**

Recovers as expected for pure acid form herbicides through hydrolysis prep steps

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Method Validation - PTs

- Soil PT result 8321 vs 8151 (Phenova, Study: HW0413)

NELAC Analyte Code	Analyte	Unit	8321A Report Value	8151A Report Value	Assigned Value	Acceptance Limit	Performance Evaluation	8321 Analysis Date
8545	2,4-D	ug/Kg	599	380	546	54.6 – 828	Acceptable	20130523
8560	2,4-DB	ug/Kg	356	155	330	33.0 – 686	Acceptable	20130523
8555	Dalapon	ug/Kg	<20	<67	<100	0.00 – 100	Acceptable	20130523
8595	Dicamba	ug/Kg	183	143	215	21.5 – 304	Acceptable	20130523
8605	Dichlorprop	ug/Kg	<10	<133	<100	0.00 – 100	Acceptable	20130523
8620	Dinoseb	ug/Kg	410	88.5	642	64.2 – 988	Acceptable	20130523
7775	MCPA	ug/Kg	<50	NA	<1000	0.00 – 1000	Acceptable	20130523
7780	MCPP	ug/Kg	<50	NA	<1000	0.00 – 1000	Acceptable	20130523
8655	2,4,5-T	ug/Kg	296	205	302	99.2 – 401	Acceptable	20130523
8650	2,4,5-TP	ug/Kg	164	85	165	16.5 - 266	Acceptable	20130523

Method Validation - PTs

More Phenova 8321 PT Study

Matrix		Water (ug/L)				Soil (ug/Kg)			
Phenova Study #		WP0114 (2/7/2014)		WP0713 (8/6/2013)		HW1013 (11/26/2013)		RR-11738 (8/6/2013)	
NELAC Analyte Code	Analyte	8321A Report Value	Assigned Value	8321A Report Value	Assigned Value	8321A Report Value	Assigned Value	8321A Report Value	Assigned Value
8545	2,4-D	3.3	2.93	8.02	8.36	360	401	703	864
8560	2,4-DB	3.1	3.10	9.02	8.26	630	683	327	286
8555	Dalapon	6.1	4.05	3.31	6.06	<9.9	<100	<10	<100
8595	Dicamba	8.7	9.09	3.84	3.44	750	881	261	251
8605	Dichlorprop	2.8	3.25	8.44	7.23	430	419	<5	<100
8620	Dinoseb	5.3	5.39	3.83	4.62	280	277	139	154
7775	MCPA	<10	<25	<10	<25	<49	<1000	<50	<1000
7780	MCPP	<10	<25	<10	<25	<49	<1000	<50	<1000
8655	2,4,5-T	6.0	6.68	3.01	3.24	220	238	428	545
8650	2,4,5-TP	9.1	8.93	8.01	8.27	580	651	625	813

PT Performance

- Passed all six PT tests on all reported analytes!
- Superior performance on 8321 herbicide method
  - ~ Excellent overall performance (over 8151) on both water and soil samples – closer to PT value
  - ~ Superior performance on 8151 poor responders
    - Dalapon – even it is a poor performer in 8321
    - MCPA, MCPP – much lower RL
    - Dinoseb in soil

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Summary

- Simple sample prep – less solvent use and no derivatization is required
- Extraction recoveries for most common herbicides are excellent and routinely fall within 30% of the true value.
- Less sample aliquot is needed – reduces sampling/shipping/disposal cost
- Faster turn-around time – 24 hours is achievable
- High sensitivity & selectivity – defendable data
- Acceptable method stability for production use
- High throughput
- Reasonable testing cost – comparable to 8151

Acknowledgements/Contacts

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Thank you

Questions?