Determination of Chlorophenoxyacetic Acid and Other Acidic Herbicides Using a QuEChERS Sample Preparation Approach and LC-MS/MS Analysis

UCT Product Number:

ECQUEU750CT-MP - Mylar pouch containing extraction salts (4000 mg $MgSO_4$, 1000 mg NaCl, 500 mg sodium citrate dibasic sesquihydrate, 1000 mg sodium citrate tribasic dihydrate)

CUMC182CT (2mL centrifuge tube containing 150 mg $MgSO_4$ and 50 mg C18)

October 2013

Chlorophenoxyacetic acid herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D; Agent Orange), along with other acidic pesticides can be extracted and analyzed using the QuEChERS approach outlined in this application note. LC-MS/MS is used for detection and quantitation.

Prior to extraction, samples need to undergo base hydrolysis to release any bound herbicide residues (in the form of esters). Hydrolysis is conducted in 30 min using a NaOH solution. The samples are subsequently neutralized using sulfuric acid, and are now ready to undergo QuEChERS extraction.

The citrate-buffered QuEChERS salts are used to lower the sample pH during extraction in order to ensure that the acidic herbicides are in their neutral state (i.e. protonated) and effectively partition into the acetonitrile layer. Only C18 sorbent is used for dSPE cleanup as the acidic compounds are otherwise retained on PSA sorbent.

Compounds included in method	Abbreviation
Picloram	-
Nitrophenol	-
Bentazon	-
2,4-dichlorophenoxyacetic acid	2,4-D
2,4-dichlorophenoxybutyric acid	2,4-DB
2-methyl-4-chlorophenoxyacetic acid	MCPA
Dichlorprop	-
2,4,5-trichlorophenoxyacetic acid	2,4,5-T
Mecoprop (MCPP)	-
Dichlorobenzoic acid	-
Silvex (fenoprop)	-
Acifluorfen	-
Thiabendazole-d_5 (IS)	TBZ-d_5

Procedure

1. Alkaline Hydrolysis

a) Weigh 10 g of thoroughly homogenized sample into a 50 mL centrifuge tube.

b) Add internal standard.

c) Add an appropriate volume of 5M NaOH in order to adjust the sample to pH 12 - this volume depends on the type and pH of the sample (e.g. 1000 µL for apples and 500 µL for tomatoes).

d) Cap tube, shake briefly, and let sit for 30 min at room temperature.

e) Neutralize the sample with the addition of 2.5M H_2SO_4 – it should use about the same volume as NaOH added (e.g. 500 µL for apples and 250 µL for tomatoes).

2. Sample Extraction

a) Add 10 mL of acetonitrile to each sample.

b) Add contents of the **ECQUEU750CT-MP** pouch to each centrifuge tube.

c) Immediately vortex or shake (manually or mechanically) for at least 1 min. For our process, samples were shaken for 1 min on a Geno/Grinder at 1500 rpm.

d) Centrifuge for 5 min at ≥3000 rcf.

3. Sample Cleanup

a) Transfer 1 mL aliquot of supernatant to 2mL **CUMC182CT** dSPE tube.

b) Vortex (or shake) for 0.5 - 1 min.

c) Centrifuge for 2 min at high rcf (e.g. ≥ 5000).

d) Filter purified supernatant through a 0.2 µm syringe filter directly into a sample vial.
e) Analyze by LC-MS/MS.

HPLC Conditions:

Instrumentation	Thermo Scientific^{TM} Dionex^{TM} Ultimate^{TM} 3000 LC system
Column	Thermo Scientific^{TM} Accucore^{TM} C18, 100 x 2.1 mm, 2.6 µm (p/n17126-102130)
Guard cartridge	Accucore^{TM} C18 Defender^{TM}, 10 x 2.1 mm, 2.6 µm (p/n17126-012105)
Run time	16 min (including re-equilibration time)
Column temperature	40°C
Injection volume	5 µL
Autosampler temperature	10°C
Wash solvent	MeOH : ultrapure water (1:1, v/v)
Flow rate	400 µL/min
Waste divert	The mobile phase was diverted to waste from 0 - 1.4 min and 12.2 - 16 min to prevent ion source contamination.

Mobile phase:		
A	0.1% formic acid in ultrapure water	
B	0.1% formic acid in MeOH	
LC gradient:		
Time (min)	**A (%)**	**B (%)**
0.0	95	5
1.0	50	50
6.0	50	50
10.0	30	70
12.2	30	70
12.5	95	5
18.0	95	5

MS Conditions:

Instrumentation	Thermo Scientific^{TM} TSQ Vantage^{TM} tandem mass spectrometer

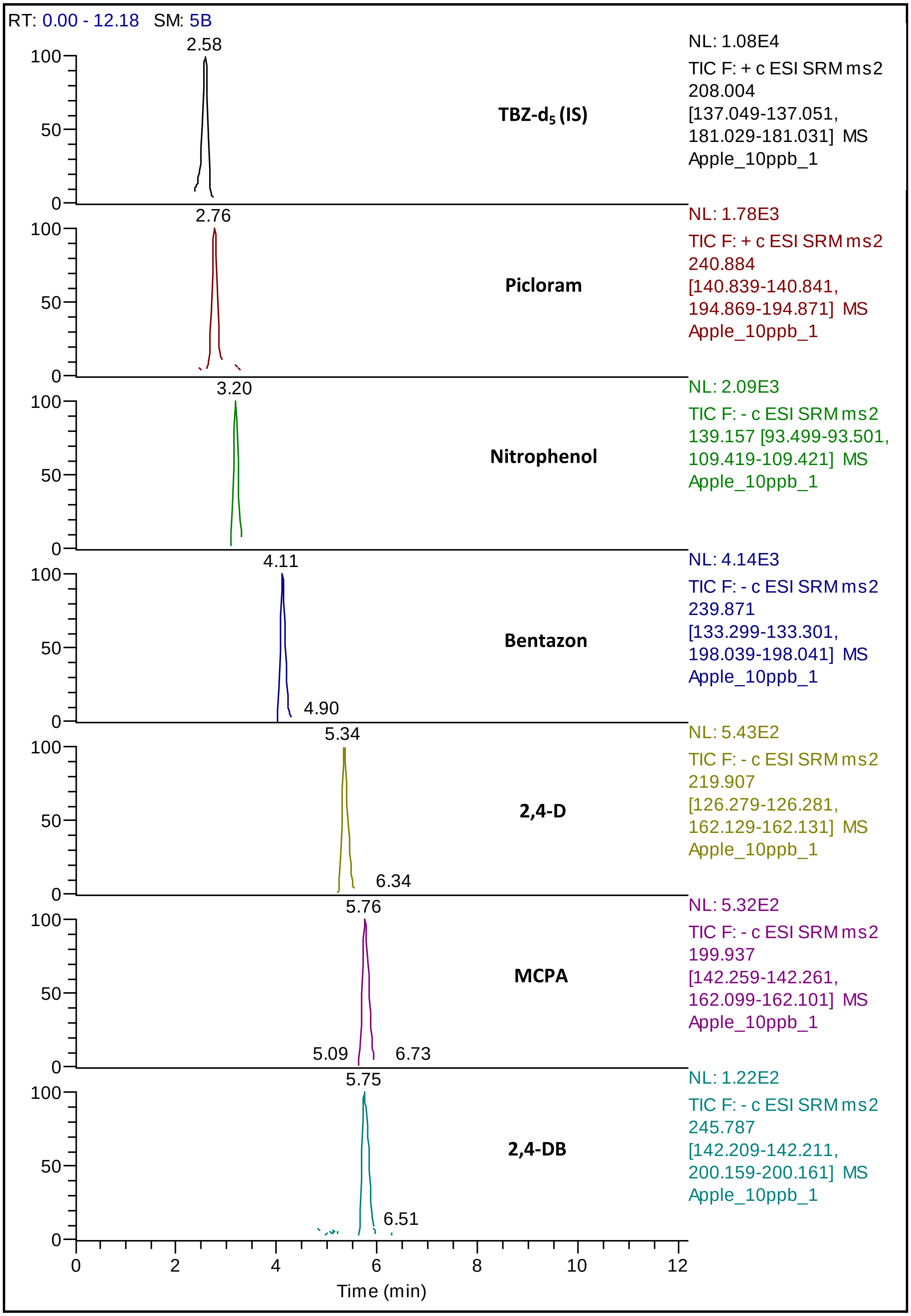
Ionization mode	ESI^+ & ESI^- with fast polarity switching
Spray voltage	5000V (ESI^+) and 4000V (ESI^-)
Vaporizer temperature	400 °C
Capillary temperature	250 °C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	45 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.1 mTorr
Acquisition method	EZ method (SRM)
Cycle time	1 sec
Software for data processing	TraceFinder^{TM} version 3.0
Weighting factor applied to calibration curves	1/X

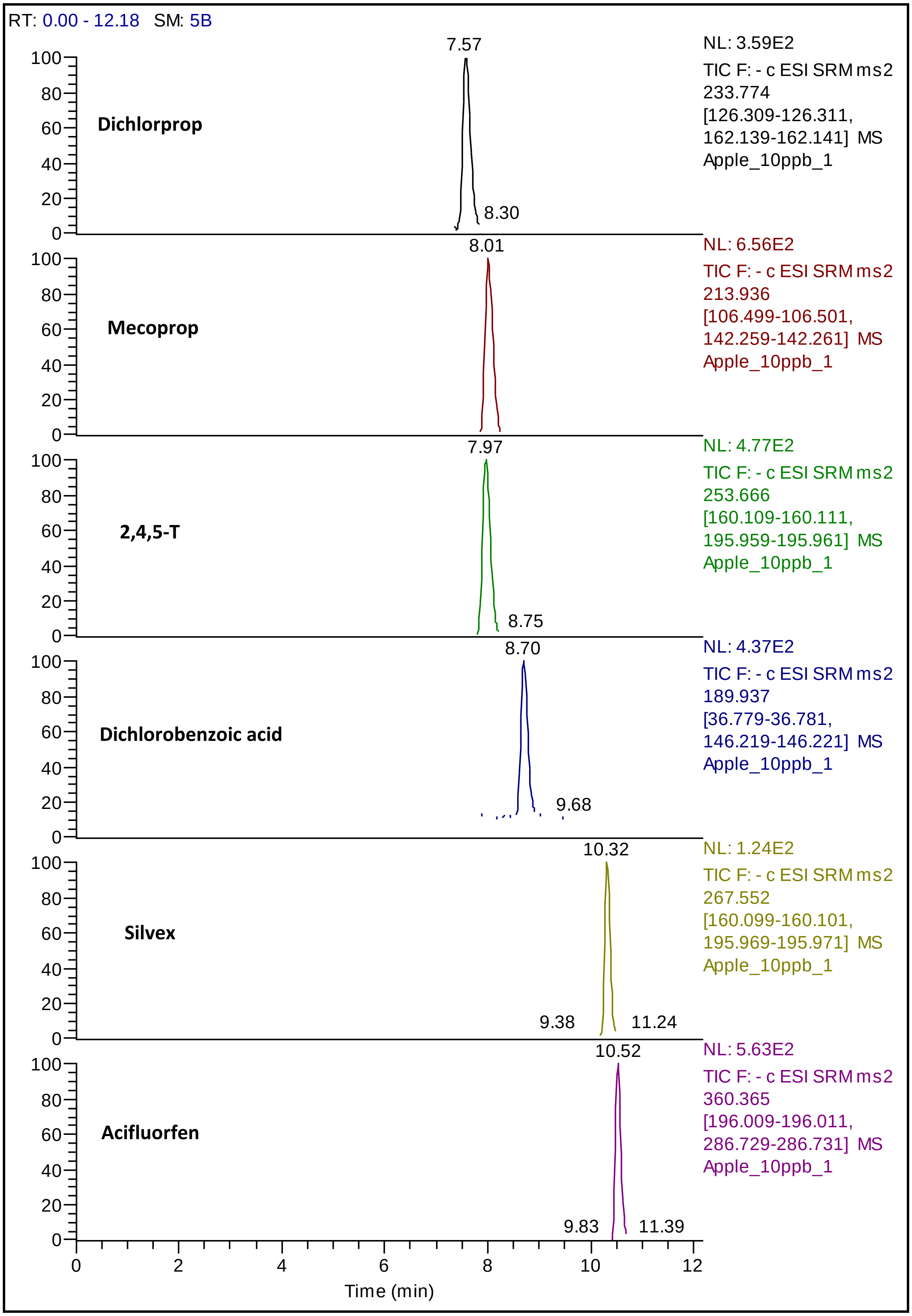
SRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
ESI^+							
TBZ-d_5 (IS)	2.58	208.0	181.0	25	137.0	31	43
Picloram	2.76	240.9	194.9	22	140.8	39	72
ESI^-							
Nitrophenol	3.17	139.2	109.4	18	93.5	24	68
Bentazon	4.11	239.9	133.3	27	198.0	21	86
2,4-D	5.34	219.9	162.1	17	126.3	30	50
2,4-DB	5.74	245.8	142.2	22	200.2	11	20
MCPA	5.74	199.9	142.3	17	162.1	20	50
Dichlorprop	7.56	233.8	162.1	17	126.3	31	50
2,4,5-T	7.95	253.7	196.0	16	160.1	28	53
Mecoprop	7.99	213.9	142.3	17	106.5	27	52
Dichlorobenzoic	8.68	189.9	146.2	13	36.8	20	54
Silvex	10.31	267.6	196.0	17	160.1	31	56
Acifluorfen	10.52	360.4	196.0	29	286.7	16	50
Dinoseb	11.13	239.8	195.1	23	135.3	47	85

Accuracy and precision data for the chlorophenoxyacetic acid herbicides at 2 concentrations.

Apple	**10 ppb (n = 6)**		**100 ppb (n = 6)**	
	Recovery (%)	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Picloram	87.3	3.1	90.6	3.7
Nitrophenol	104.2	4.2	103.2	5.0
Bentazone	86.6	2.7	91.4	4.0
2,4-D	89.6	3.9	96.4	3.4
MCPA	90.8	3.7	100.8	5.2
2,4-DB	90.3	9.9	99.4	3.7
Dichlorprop	95.0	2.9	96.3	4.8
2,4,5-T	87.3	5.0	97.1	2.7
Mecoprop	84.0	1.4	94.7	0.7
Dichlorobenzoic acid	86.6	4.6	89.6	0.7
Silvex	101.6	6.9	102.7	6.3
Acifluorfen	88.5	3.2	94.8	3.0
Overall average	***91.0***	***4.3***	***96.4***	***3.6***

Tomato	**10 ppb (n = 6)**		**100 ppb (n = 6)**	
	Recovery (%)	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Picloram	90.3	2.1	88.7	3.1
Nitrophenol	93.2	8.0	97.3	3.2
Bentazone	85.5	3.2	92.4	1.8
2,4-D	91.5	3.3	97.9	1.9
MCPA	94.3	4.8	105.2	3.5
2,4-DB	101.5	4.3	100.8	2.9
Dichlorprop	98.4	5.6	102.2	5.0
2,4,5-T	95.4	4.7	104.2	3.7
Mecoprop	88.2	1.6	97.1	2.2
Dichlorobenzoic acid	97.4	2.6	98.2	1.3
Silvex	101.8	8.5	111.0	6.7
Acifluorfen	101.2	7.3	99.8	2.9
Overall average	***94.9***	***4.7***	***99.6***	***3.2***


RT: 0.00 - 12.18 SM: 5B
2.58
NL: 1.08E4
TIC F: + c ESI SRM ms2 208.004 [137.049-137.051, 181.029-181.031] MS Apple_10ppb_1
TBZ-d5 (IS)
2.76
NL: 1.78E3
TIC F: + c ESI SRM ms2 240.884 [140.839-140.841, 194.869-194.871] MS Apple_10ppb_1
Picloram
3.20
NL: 2.09E3
TIC F: - c ESI SRM ms2 139.157 [93.499-93.501, 109.419-109.421] MS Apple_10ppb_1
Nitrophenol
4.11
4.90
NL: 4.14E3
TIC F: - c ESI SRM ms2 239.871 [133.299-133.301, 198.039-198.041] MS Apple_10ppb_1
Bentazon
5.34
6.34
NL: 5.43E2
TIC F: - c ESI SRM ms2 219.907 [126.279-126.281, 162.129-162.131] MS Apple_10ppb_1
2,4-D
5.76
5.09
6.73
NL: 5.32E2
TIC F: - c ESI SRM ms2 199.937 [142.259-142.261, 162.099-162.101] MS Apple_10ppb_1
MCPA
5.75
6.51
NL: 1.22E2
TIC F: - c ESI SRM ms2 245.787 [142.209-142.211, 200.159-200.161] MS Apple_10ppb_1
2,4-DB
100
50
0
0
2
4
6
8
10
12
Time (min)



Chromatogram: Apple sample spiked with 10 ng/g chlorophenoxyacetic acid herbicides.