Determination of Pesticide Residues in Blueberries by AOAC QuEChERS Approach and Dispersive SPE Cleanup with a Novel Sorbent ChloroFiltr®

UCT Part Numbers:
ECMSSA50CT-MP - Mylar pouch containing 6 g $MgSO_4$ and 1.5 g NaOAc with 50-mL centrifuge tubes included
CUMPSGGC182CT - 2 mL centrifuge tube containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 50 mg ChloroFiltr®
SLAQ100ID21-3UM - Selectra® Aqueous C18, 100 x 2.1 mm, 3 µm
SLAQGDC20-3UM - Selectra® Aqueous C18, Guard column, 10 x 2.1 mm, 3 µm
SLGRDHLDR - Guard Cartridge Holder

Summary:

Blueberry has been ranked as one of the healthiest fruits for its high antioxidant content that helps combating free radicals, which could damage DNA and cellular structures [1]. Application of pesticides during plant cultivation is common to increase product yield, therefore it is valuable developing effective analytical methods for the determination of pesticide residues in blueberries, which however is challenging due to matrix complexity as blueberries are rich in anthocyanins, sugars, polyphenols, vitamins, minerals, and other interfering components.

This application outlines a simple, fast, and cost-effective method for the determination of multi-class pesticides, including one of the most problematic pesticides, pymetrozine in blueberries. The acetate buffered AOAC QuEChERS protocol demonstrated higher extraction efficiency for pymetrozine than the other 2 QuEChERS protocols (the EN citrate buffered or the original unbuffered), thus was selected for the extraction of pesticide residues in blueberries. 15 g of homogenized blueberries were extracted with 15 mL of acetonitrile (MeCN) containing 1% acetic acid (HAc). 6 g magnesium sulfate ($MgSO_4$) and 1.5 g sodium acetate (NaOAc) were employed to enhance phase separation and partition of pesticides into the MeCN layer. After shaking and centrifugation, 1 mL of the supernatant was transferred to a 2-mL dSPE tube containing the optimized cleanup sorbents of 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 50 mg

ChloroFiltr®. Residual water was absorbed by $MgSO_4$, anthocynins, polyphenols, sugars and organic acids were removed by PSA, lipids and other non-polar interferences were retained by C18, while chlorophylls were removed by ChloroFiltr®, resulting in clean extract for LC/MS/MS analysis. UCT's aqueous C18 HPLC column was used for analyte retention and separation, which showed superior retention especially for several very polar pesticides, such as methamidophos and acephate.

Procedure:

1. **QuEChERS extraction**
    a) Weigh 15 ± 0.3 g of homogenized blueberry sample into 50-mL centrifuge tubes.
    b) Add triphenyl phosphate (TPP) as internal standard (IS) (optional), and appropriate amounts of spiking solution to fortified samples.
    c) Add 15 mL of MeCN with 1% HAc. Cap and shake for 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
    d) Add salts (6 g $MgSO_4$ and 1.5 g NaOAc) from pouch (**ECMSSA50CT-MP**) to the 50-mL tube, and vortex for 10 sec to break up salt agglomerates.
    e) Shake for 1 min at 1000 strokes/min using the Geno-Grinder.
    f) Centrifuge at 3000 rcf for 5 min.

2. **dSPE cleanup**
    a) Transfer 1 mL of the supernatant to a 2-mL dSPE tube (**CUMPSGGC182CT**).
    b) Shake for 1 min at 1000 strokes/min using the Spex 2010 Geno-Grinder.
    c) Centrifuge at 3000 rcf for 5 min.
    d) Transfer 0.2 mL of the cleaned extract into a 2-mL auto-sampler vial, add 0.2 mL of reagent water, and vortex for 30 sec.
    e) The samples are ready for LC-MS/MS analysis.

LC-MS/MS Method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 2 µL		
Gradient program:		
Time (min)	**A%** (10 mM ammonium acetate in DI water)	**B%** (0.1% formic acid in MeOH)
0	100	0
1	50	50
3.5	50	50
6	5	95
9	5	95
9.1	100	0
14	100	0

Divert mobile phase to waste from 0 – 1.5 and 11.5 – 14 min to prevent ion source contamination.

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	3500 V
Vaporizer temperature	450 °C
Ion transfer capillary	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	40 arbitrary units
Q1 and Q3 peak width	0.4 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.5 sec
Acquisition method	EZ Method (scheduled SRM)

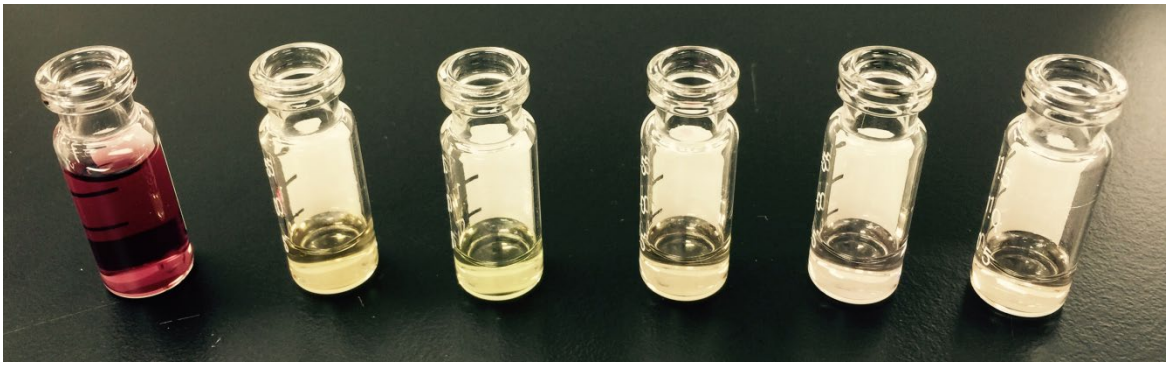
SRM Table						
Compound	**Precursor**	**Product 1**	**CE1**	**Product 2**	**CE2**	**S-lens RF**
Metamidophos	142.0	94.1	14	125.0	13	50
Acephate	184.0	143.0	6	95.0	25	33
Aldicarb sulfoxide	207.1	89.1	13	69.1	16	32
Oxydemeton methyl	247.0	169.0	13	109.0	27	57
Pymetrozine	218.1	105.1	20	176.1	17	63
Dichrotophos	238.1	112.1	12	127.0	18	52
Triethylphosphorothioate	199.0	125.0	16	143.0	14	55
Dimethoate	230.0	125.0	22	171.0	15	50
Carbendazim	192.1	160.1	18	132.1	29	60
Dichlorvos	220.9	109.0	17	127.0	13	62
Thiabendazole	202.0	175.1	25	131.1	31	70
Fenamiphos sulfone	336.1	266.0	19	188.0	26	75
Fenamiphos sulfoxide	320.1	233.0	24	108.1	40	60
Simazine	202.1	132.0	19	124.1	16	66
Tebuthiuron	229.1	172.1	16	116.0	26	55
Carbaryl	202.1	145.1	11	127.1	30	38
Flutriafol	302.1	70.1	17	123.0	28	69
Famphur	326.0	217.0	20	93.0	30	68
Thionazin	249.0	113.0	23	97.0	28	58
DEET	192.1	119.1	17	91.1	29	64
Atrazine	216.1	174.1	16	68.1	34	66
Malathion	331.0	127.0	12	99.0	25	55
Triadimefon	294.1	197.1	14	69.1	20	65
Pyrimethanil	200.1	107.1	24	183.1	23	68
Acetochlor	270.1	224.1	10	148.1	18	58
Sulfotep	323.0	97.0	37	115.0	30	60
Tebuconazole	308.1	70.1	21	125.0	33	66
Zoxamide	336.0	187.0	21	159.0	38	74
Diazinon	305.1	169.1	20	153.1	20	68
TPP (IS)	327.1	152.1	35	77.1	38	95
Cyprodinil	226.1	93.1	33	77.1	43	70
Pyrazophos	374.1	222.1	20	194.1	31	100
Profenofos	372.9	302.9	17	128.0	42	73
Ethion	385.0	142.9	26	199.0	6	56
Chlorpyrifos	349.9	97.0	32	197.9	19	67

Results:

Selection of dSPE cleanup sorbents:

Different sorbent mixtures (A - E) were packed in 2-mL dSPE centrifuge tubes for blueberry extract cleanup:

A. 150 mg $MgSO_4$ and 50 mg PSA
B. 150 mg $MgSO_4$ and 150 mg PSA
C. 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18
D. 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 7.5 mg GCB
E. 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 50 mg ChloroFiltr®



Photographs, from left to right: crude blueberry extract, and extracts cleaned with sorbent mixture A, B, C, D, and E, respectively.

Illustrated in the above picture, cleanup of blueberry extracts with PSA only (A and B) or PSA and C18 (C) is inefficient for complete pigment removal. With the addition of either GCB (D) or ChloroFiltr® (E), colorless extracts were obtained; therefore sorbent mixtures D and E were selected for the recovery study.

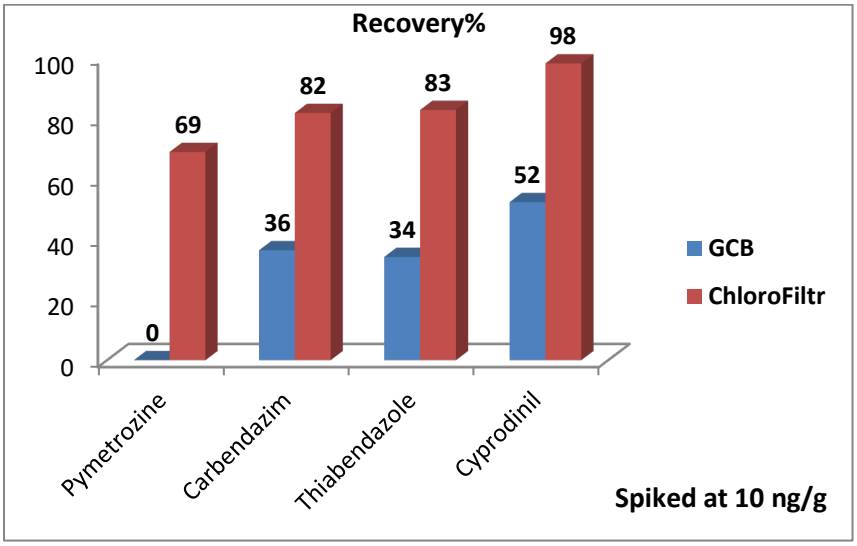
Recovery study:

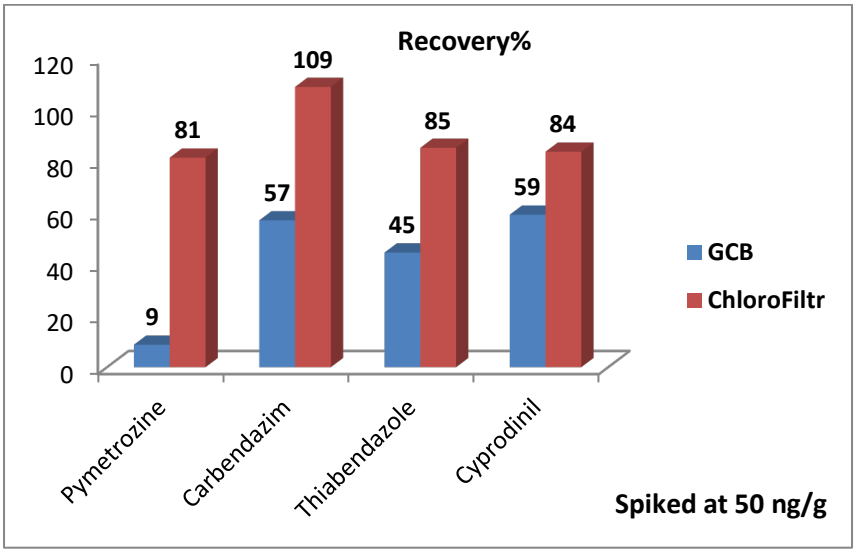
Blueberry samples were fortified with 10 ng/g and 50 ng/g of pesticides, and underwent the AOAC QuEChERS extraction and dSPE cleanup with 150 mg MgSO4, 50 mg PSA, 50 mg C18, and 7.5 mg GCB (D) or 50 mg ChloroFiltr® (E) as described above. The mean recoveries and RSD% of 6 replicated samples are listed in the table below.

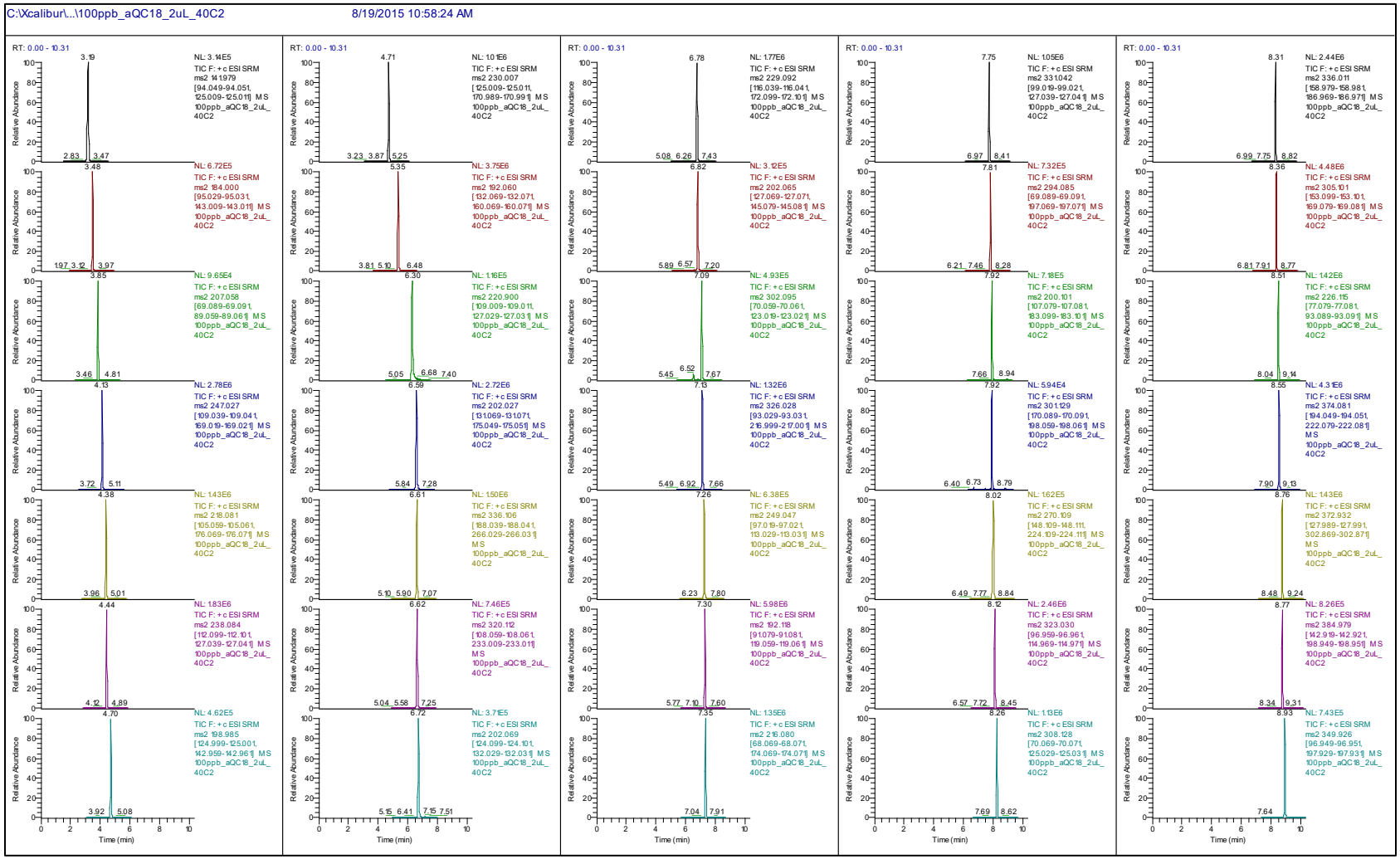
Accuracy and Precision of Pesticides in Spiked Blueberries

Compound	dSPE cleanup with PSA/C18/GCB				dSPE cleanup with PSA/C18/ChloroFiltr®			
	Spiked at 10 ng/g		Spiked at 50 ng/g		Spiked at 10 ng/g		Spiked at 50 ng/g	
	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%
Methamidophos	**82.8**	2.5	**92.8**	1.0	**85.0**	3.7	**89.3**	1.2
Acephate	**82.8**	11.0	**83.1**	12.8	**86.1**	11.3	**89.5**	13.3
Aldicarb_sulfoxide	**87.0**	6.5	**95.6**	12.7	**103.4**	11.5	**82.1**	12.9
Oxydemeton_methyl	**96.6**	5.9	**94.0**	7.4	**96.2**	5.9	**84.2**	8.2
Dichrotophos	**65.7**	20.1	**103.6**	10.7	**91.6**	13.3	**102.3**	15.6
Pymetrozine	**0.0**	na	**8.7**	9.4	**68.8**	11.6	**81.3**	13.4
Dimethoate	**62.8**	16.1	**103.0**	6.2	**101.1**	10.9	**94.9**	10.6
Triethylphosphorothioate	**65.0**	11.9	**99.9**	6.7	**96.4**	12.5	**95.8**	10.4
Carbendazim	**36.3**	9.9	**57.0**	11.1	**81.7**	5.4	**108.7**	9.5
Dichlorvos	**91.0**	6.1	**103.3**	1.8	**95.4**	4.9	**91.5**	3.8
Fenamiphos_sulfone	**96.1**	1.9	**104.1**	1.6	**97.2**	5.5	**97.9**	9.4
Fenamiphos_sulfoxide	**94.5**	2.3	**99.2**	1.4	**101.0**	4.2	**94.9**	10.5
Simazine	**94.5**	7.3	**103.7**	3.1	**99.4**	8.0	**94.5**	6.0
Carbaryl	**103.5**	3.3	**104.2**	3.1	**95.4**	3.7	**98.7**	6.0
Tebuthiuron	**95.6**	2.0	**101.1**	1.9	**97.9**	1.9	**97.7**	6.7
Thiabendazole	**34.1**	7.0	**44.5**	7.7	**82.7**	2.9	**85.1**	9.8
Famphur	**103.3**	3.1	**109.4**	1.4	**98.1**	10.0	**102.7**	1.7
Flutriafol	**96.4**	2.3	**105.9**	0.9	**92.1**	3.5	**97.4**	1.4
Thionazin	**104.3**	2.8	**105.6**	1.8	**90.8**	14.9	**97.6**	4.3
Atrazine	**116.9**	2.7	**116.1**	1.8	**84.6**	12.3	**86.6**	11.5
DEET	**127.6**	3.8	**112.9**	2.5	**85.1**	24.9	**84.7**	18.8
Malathion	**94.5**	7.5	**108.2**	2.0	**95.4**	0.9	**104.2**	3.7
Triadimefon	**89.9**	3.3	**104.3**	2.0	**91.4**	5.5	**99.0**	1.6
Pyrimethanil	**62.6**	9.3	**72.8**	3.8	**81.9**	5.9	**89.3**	2.3
Acetochlor	**95.7**	3.7	**105.2**	2.8	**102.1**	5.7	**97.8**	2.4
Sulfotep	**94.2**	3.3	**107.7**	1.7	**96.3**	3.0	**106.3**	1.2
Tebuconazole	**93.0**	3.5	**102.6**	1.7	**87.3**	2.1	**93.5**	1.8
Zoxamide	**99.0**	2.5	**109.1**	1.1	**89.1**	2.1	**96.3**	2.2
Diazinon	**93.8**	2.4	**103.1**	0.9	**93.4**	3.5	**96.4**	1.1
Cyprodinil	**52.2**	5.9	**59.2**	6.6	**98.1**	3.4	**83.6**	1.7
Pyrazophos	**70.2**	5.5	**76.1**	7.5	**94.5**	2.0	**99.3**	1.3
Ethion	**96.8**	4.0	**100.9**	6.1	**95.9**	3.3	**93.6**	2.0
Profenofos	**91.0**	3.6	**98.9**	3.9	**88.1**	2.5	**87.3**	1.6
Chlorpyrifos	**88.7**	2.1	**98.5**	3.4	**94.6**	0.9	**89.1**	2.2

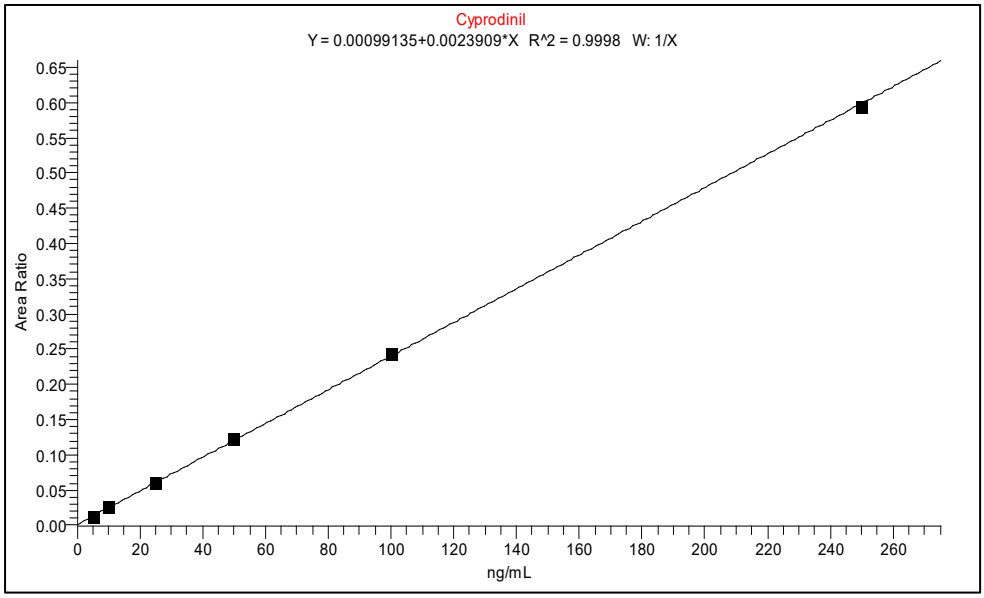
The recoveries of several pesticides such as pymetrozine, carbendazim, thiabendazole, and cyprodinil, were found be adversely affected by GCB, but unaffected when ChloroFiltr® was used; therefore, this sorbent combination was selected for blueberry extract cleanup in the final optimized procedure. The graphs below demonstrate the recovery comparison using GCB versus ChloroFiltr® at 2 contrasting levels (10 and 50 ng/g).







Chromatograms of 34 Pesticides and TPP (IS) in 1:1 MeCN:H_2O (100 ppb) using UCT Aqueous C18 HPLC Column
(Compound order can be found in the SRM table.)



Matrix-matched Calibration Curve of Cyprodinil (R^2 = 0.9998)

References:

[1] http://www.whfoods.com/genpage.php?tname=foodspice&dbid=8

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • www.unitedchem.com • Email: methods@unitedchem.com