Pesticide Residue Analysis in Whole Milk by QuEChERS and LC-MS/MS

UCT Part Numbers:
Enviro-Clean® RFV0050CT (50 mL centrifuge tubes)
Enviro-Clean® ECMSSA50CT-MP (Mylar pouch containing 6 g $MgSO_4$ and 1.5 g NaOAc)
Enviro-Clean® CUMPSC18CT (2 mL dSPE tube with 150 mg MgSO4, 50 mg PSA and 50 mg C18)

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Summary:

This application describes a cost-effective and easy to use method for the determination of pesticide residues in whole milk. The method employs the AOAC version of QuEChERS. This procedure provides better analytical results than either the original or EN versions of the QuEChERS procedure in extracting a few sensitive pesticides; such as pymetrozine and hexazinone (Velpar).

15 mL of whole milk is extracted using 15 mL of acetonitrile (MeCN) with 1%(v/v) acetic acid (HAc); 6 g magnesium sulfate ($MgSO_4$) and 1.5 g sodium acetate (NaOAc) are added into the mixture to enhance the phase separation and the extraction of pesticides. After shaking and centrifugation, 1 mL of the supernatant is purified by 2-mL dSPE tube containing 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18. $MgSO_4$ absorbs residual water in the extract, PSA removes organic acids and carbohydrates, while C18 retains fatty acids and cholesterol. The pesticides in the cleaned extract are detected and quantified by LC-MS/MS.

Matrix matched calibration curves were constructed for pesticide quantification. The responses for all 24 pesticides were linear with R^2 ranged from 0.9954 to 0.9997 over the concentration range of 2 to 400 ng/mL. Excellent recoveries and relative standard deviations were obtained, indicating that this method is suitable for pesticide analysis in whole milk samples, especially when pymetrozine and hexazinone are being analyzed.

Procedure:

1. **QuEChERS extraction**
    a) Transfer 15 mL of whole milk into 50-mL centrifuge tube (**RFV0050CT**).
    b) Add 30 µL of 50-ppm triphenyl phosphate (TPP) internal standard (IS) solution to all samples, and appropriate amounts of 2 ppm pesticide working solution to fortified samples.
    c) Add 15 mL of MeCN with 1% HAc. Cap and shake 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
    d) Add salts, 6 g MgSO4 and 1.5 g NaOAc from pouch (**ECMSSA50CT-MP**), and vortex for 10 sec to break up salt agglomerates.
    e) Shake 1 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
    f) Centrifuge at 3830 rcf for 5 min.

2. **dSPE cleanup**
    a) Transfer 1 mL of the supernatant to 2-mL dSPE tube (**CUMPSC18CT**).
    b) Shake 2 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
    c) Centrifuge at 15,300 rcf for 5 min.
    d) Transfer 0.3 mL of the cleaned extract into 2-mL auto-sampler vial, add 0.3 mL of reagent water, and vortex for 30 sec.
    e) The samples are ready for LC-MS/MS analysis.

Whole Milk Samples Extracted by the AOAC QuEChERS Procedure

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: Thermo Scientific, Accucore aQ®, 100 x 2.1 mm, 2.6 µm		
Guard Column: Thermo Scientific, Accucore aQ®, 10 x 2.1 mm, 2.6 µm		
Column Temperature: 40 °C		
Column Flow Rate: 0.200 mL/min		
Auto-sampler Temperature: 10 °C		
Injection Volume: 10 µL		
Gradient Program: **Mobile Phase A:** 0.3 % formic acid and 0.1 % ammonia formate in water **Mobile Phase B:** 0.1 % formic acid in MeOH		
Time (min)	**Mobile Phase A (%)**	**Mobile Phase B (%)**
0	99	1
1.5	99	1
3.5	20	80
10	10	90
12	0	100
15	0	100
15.2	99	1
20	99	1
Divert mobile phase to waste from 0 - 0.5 and 15 - 20 min to prevent ion source contamination.		

MS parameters	
Polarity	ESI +
Spray voltage V	4000 V
Vaporizer Temperature	300 °C
Ion transfer capillary	200 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Scan type	SRM
Cycle time	1 sec
Acquisition method	EZ Method

SRM transitions

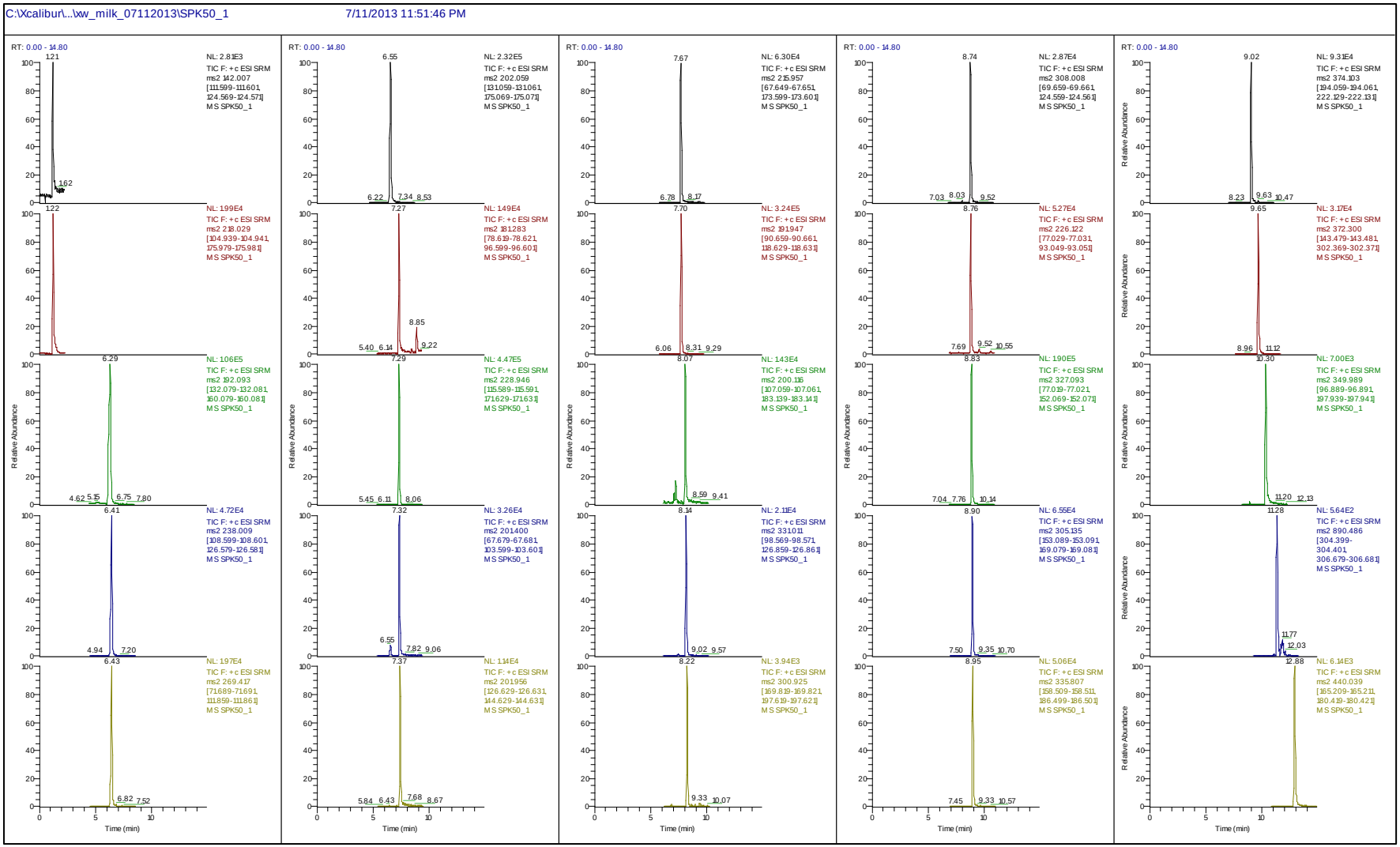
Name	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Methamidophos	1.21	142.007	124.57	14	111.6	5	60
Pymetrozine	1.22	218.029	104.94	18	175.98	16	70
Carbendazim	6.29	192.093	160.08	17	132.08	29	81
Dicrotophos	6.41	238.009	126.58	17	108.60	33	73
Acetachlor	6.43	269.417	111.86	15	71.69	33	72
Thiabendazole	6.55	202.059	175.07	24	131.06	31	103
DIMP	7.27	181.283	96.60	13	78.62	32	44
Tebuthiuron	7.29	228.946	171.63	17	115.59	26	72
Simazine	7.32	201.400	67.68	33	103.60	24	85
Carbaryl	7.37	201.956	144.63	7	126.63	30	40
Atrazine	7.67	215.957	173.60	16	67.65	35	79
DEET	7.70	191.947	118.63	15	90.66	28	92
Pyrimethanil	8.07	200.116	107.06	23	183.14	22	66
Malathion	8.14	331.011	126.86	12	98.57	23	60
Bifenazate	8.22	300.925	169.82	15	197.62	5	51
Tebuconazole	8.74	308.008	69.66	29	124.56	35	97
Cyprodinil	8.76	226.122	93.05	33	77.03	40	88
TPP (IS)	8.83	327.093	152.07	33	77.02	37	98
Diazinon	8.90	305.135	169.08	14	153.09	15	89
Zoxamide	8.95	335.807	158.51	38	186.50	20	102
Pyrazophos	9.02	374.103	222.13	20	194.06	20	104
Profenofos	9.65	372.300	302.37	19	143.48	35	104
Chlorpyrifos	10.30	349.989	197.94	17	96.89	32	69
Abamectin	11.28	890.486	304.40	18	306.68	15	102
Bifenthrin	12.88	440.039	180.42	11	165.21	39	66

Results:

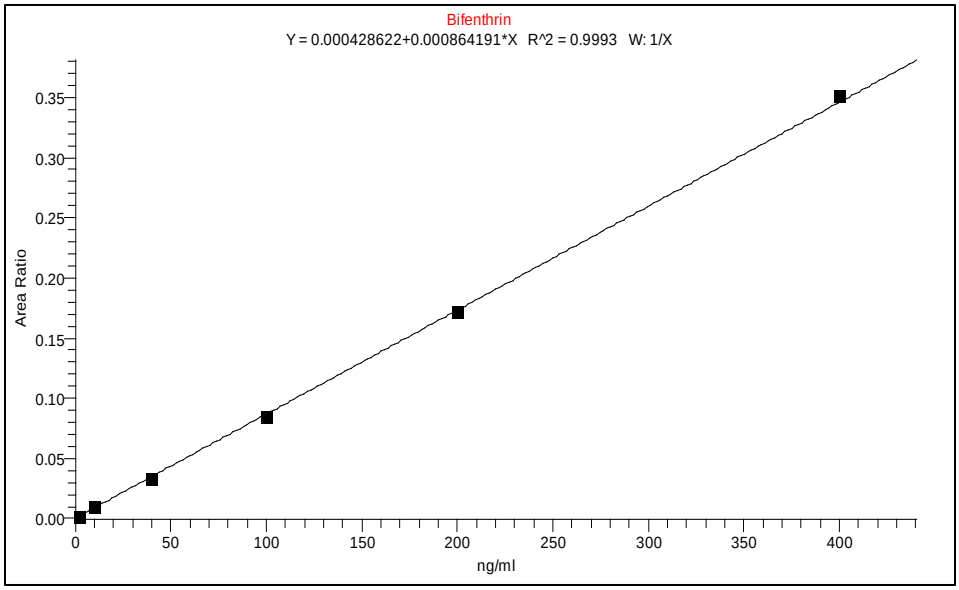
Recovery and RSD% Obtained from 5 Replicated Fortified Milk Samples

Analytes	Spiked at 10 ng/mL		Spiked at 50 ng/mL	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)
Methamidophos	**85.2**	5.8	**100.3**	5.1
Pymetrozine	**93.9**	5.2	**97.3**	5.4
Carbendazim	**100.4**	3.8	**102.8**	3.1
Dicrotophos	**102.3**	2.1	**106.5**	2.9
Acetachlor	**119.9**	3.6	**128.8**	2.9
Thiabendazole	**99.8**	2.1	**103.8**	2.3
DIMP	**90.3**	3.2	**93.1**	4.7
Tebuthiuron	**108.6**	3.0	**113.3**	2.7
Simazine	**102.6**	1.6	**105.1**	2.7
Carbaryl	**95.6**	5.3	**97.1**	4.0
Atrazine	**99.1**	2.0	**102.8**	3.0
DEET	**103.6**	2.4	**106.4**	3.4
Pyrimethanil	**91.0**	4.7	**92.3**	4.0
Malathion	**100.7**	3.8	**99.1**	3.0
Bifenazate	**85.6**	9.1	**81.0**	8.7
Tebuconazole	**91.0**	2.7	**91.9**	3.5
Cyprodinil	**94.2**	2.1	**95.6**	3.1
Diazinon	**96.8**	2.6	**97.7**	3.5
Zoxamide	**100.4**	3.0	**101.9**	3.0
Pyrazophos	**100.3**	1.6	**104.0**	2.0
Profenofos	**90.9**	2.8	**93.0**	3.9
Chlorpyrifos	**94.2**	4.9	**87.8**	4.5
Abamectin	**81.3**	7.7	**86.6**	4.2
Bifenthrin	**77.8**	3.1	**75.8**	2.1
Overall mean	**96.1**	3.7	**98.5**	3.7

Chromatograms of a Fortified Whole Milk Sample at 50 ng/mL



Matrix Matched Calibration Curves of Bifenthrin (R^2=0.9993)



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