

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



## UCT Part Numbers

**ECPSACB256**  
500 mg PSA,  
250 mg GCB/6 mL cartridge

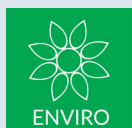
**ECMSSC50CT-MP**  
4000 mg MgSO<sub>4</sub>  
1000 mg NaCl

## 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<sub>4</sub>) 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<sub>4</sub>, 50 mg PSA, and 50 mg C18. MgSO<sub>4</sub> 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<sup>2</sup> 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

- Transfer 15 mL of whole milk into 50-mL centrifuge tube (RFV0050CT).
- Add 30  $\mu$ L 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.
- Add 15 mL of MeCN with 1% HAC. Cap and shake 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
- Add salts, 6 g  $\text{MgSO}_4$  and 1.5 g NaOAc from pouch (ECMSSA50CT-MP), and vortex for 10 sec to break up salt agglomerates.
- Shake 1 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
- Centrifuge at 3830 rcf for 5 min.

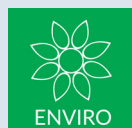
### 2. dSPE Cleanup

- Transfer 1 mL of the supernatant to 2-mL dSPE tube (CUMPSC18CT).
- Shake 2 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
- Centrifuge at 15,300 rcf for 5 min.
- 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.
- The samples are ready for LC-MS/MS analysis.



Whole Milk Samples Extracted by the AOAC QuEChERS Procedure

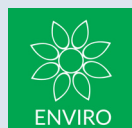
LC-MS/MS Method	
HPLC	Thermo Scientific Dionex UltiMate 3000® LC System
Column	Thermo Scientific, Accucore aQ®, 100 x 2.1 mm, 2.6 $\mu$ m
Guard Column	Thermo Scientific, Accucore aQ®, 10 x 2.1 mm, 2.6 $\mu$ m
Column Temperature	40 °C
Column Flow Rate	0.200 mL/min
Auto-sampler Temperature	10 °C
Injection Volume	10 $\mu$ L
Gradient Program	<b>Mobile Phase A:</b> 0.3 % formic acid and 0.1 % ammonia formate in water <b>Mobile Phase B:</b> 0.1 % formic acid in MeOH



Gradient Program		
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 <sup>+</sup>
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



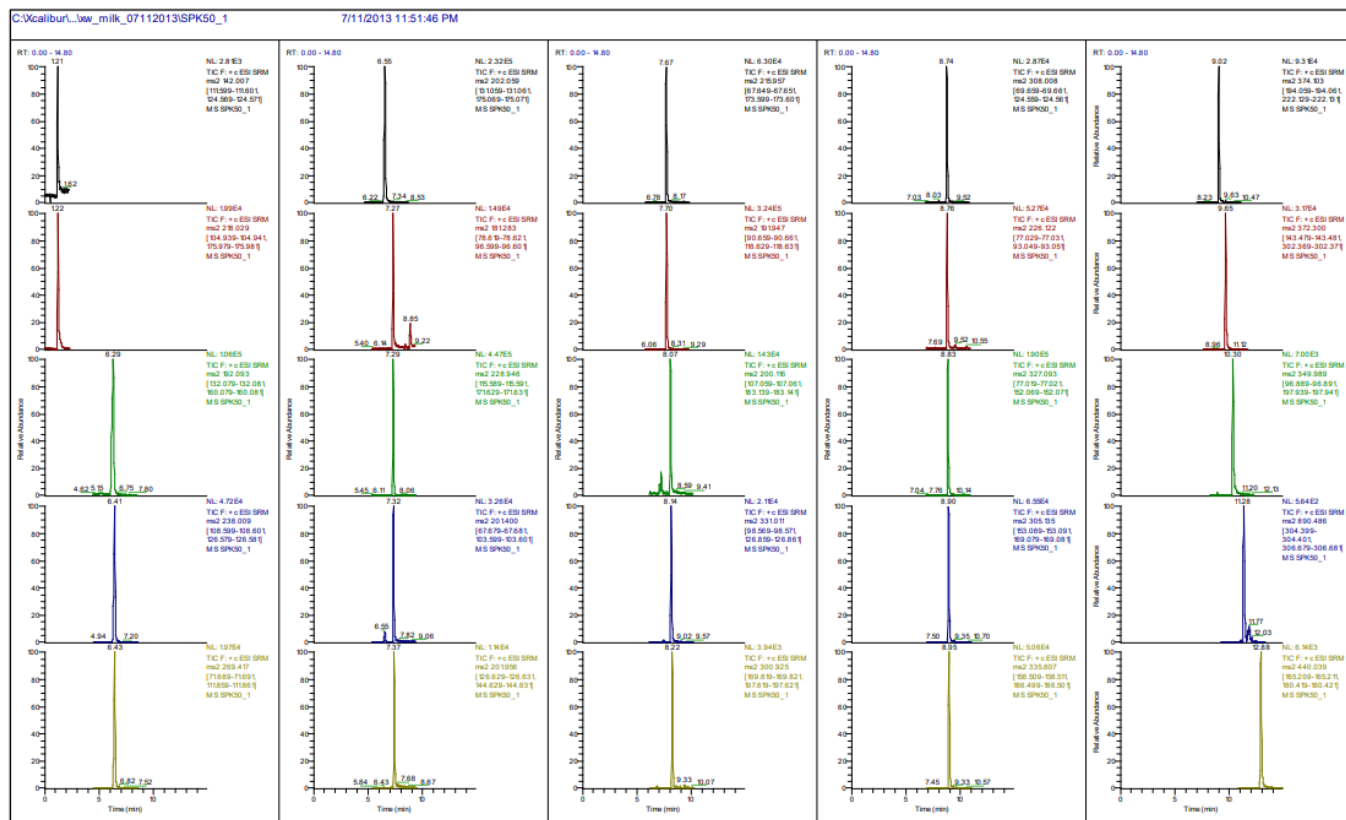
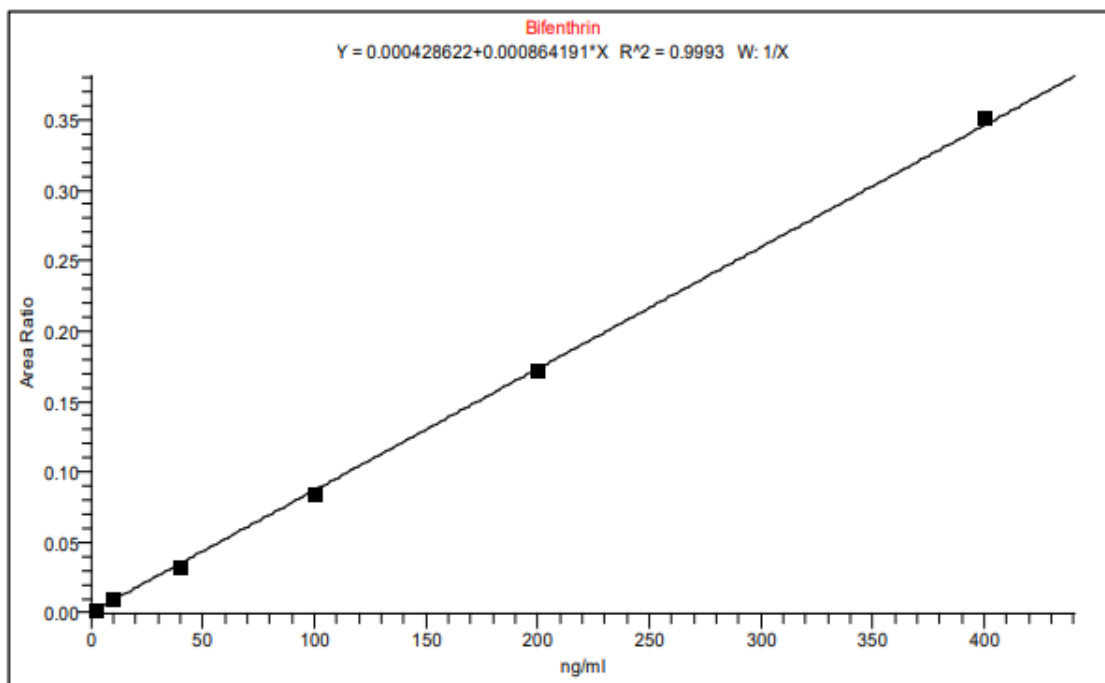
SRM Transitions							
Name	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Methamidophos	1.21	142.007	124.57	14	111.6	5	60
Pymetrozine	1.22	218.029	104.94	18	175.98	16	70
Carbendazim	6.29	192.093	160.08	17	132.08	29	81
Dicrotophos	6.41	238.009	126.58	17	108.60	33	73
Acetachlor	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				
	Spiked at 10 ng/mL		Spiked at 50 ng/mL	
Analytes	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
Diclotophos	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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