



Determination of Pesticides in Bananas 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 MgSO₄ 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 simple, fast, and cost-effective method for the determination of multi-class pesticides in bananas including one of the most difficult compounds, pymetrozine. The method employs the AOAC version of the QuEChERS procedure, in which 15 g of the homogenized banana sample is hydrated with 5 mL of reagent water to give a sample with > 80% water content. The hydrated sample is extracted by 15 mL of acetonitrile (MeCN) with 1% (v/v) acetic acid (HAc), followed by the addition of 6 g anhydrous magnesium sulfate (MgSO₄) and 1.5 g sodium acetate (NaOAc). After shaking and centrifugation, 1 mL of the supernatant is transferred to a 2-mL dSPE tube containing 150 mg MgSO₄, 50 mg PSA, and 50 mg C18. The MgSO₄ absorbs residual water; PSA removes organic acids and sugars, while the C18 removes fatty acids and other non-polar interferences in the sample. The result is a clean extract for LC-MS/MS analysis.

Matrix matched calibration curves were constructed for pesticide quantification. The responses for all 24 pesticides were linear with R² ranging from 0.9939 to 0.9998 over the concentration range of 2 to 400 ng/g.

Recoveries were excellent with an average recovery of 97% and an average relative standard deviation of about 6.25% using the data from spiked matrix samples with concentrations of 10ng/g and 50ng/g.

The results from this application indicate this pesticide method is suitable in bananas especially when pymetrozine is required to be analyzed.

Procedure:

1. QuEChERS extraction

- a) Weigh 15 ± 0.15 g of peeled and homogenized banana sample into a 50-mL centrifuge tube (**RFV0050CT**). Prepare 5 fortified samples, each at two spiking levels.
- b) Add 5 mL of reagent water to each tube (to increase the water content in banana from 74% to > 80%).
- c) Add 30 μ 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.
- d) Add 15 mL of MeCN with 1%(v/v) HAc. Cap and shake for 1 min at 1000 strokes/min using a Spex 2010 Geno-Grinder.
- e) Add salts (6 g MgSO₄ and 1.5 g NaOAc) from pouch (**ECMSSA50CT-MP**), and vortex for 10 sec to break up any salt agglomerates.
- f) Shake for 1 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
- g) Centrifuge at 5000 rpm (or 3830 rcf) for 5 min.

2. dSPE cleanup

- a) Transfer 1 mL of the supernatant to 2 mL dSPE tube (**CUMPSC18CT**).
- b) Shake for 2 min at 1000 strokes/min using Spex 2010 Geno-Grinder.
- c) Centrifuge at 10,000 rpm (or 15,300 rcf) for 5 min.
- d) Transfer 0.3 mL of the cleaned extract into a 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.

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000 [®] LC System		
Column: Thermo Scientific, Accucore aQ [®] , 100 x 2.1 mm, 2.6 μ m		
Guard Column: Thermo Scientific, Accucore aQ [®] , 10 x 2.1 mm, 2.6 μ m		
Column Temperature: 40 °C		
Column Flow Rate: 0.200 mL/min		
Auto-sampler Temperature: 10 °C		
Injection Volume: 10 μ L		
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

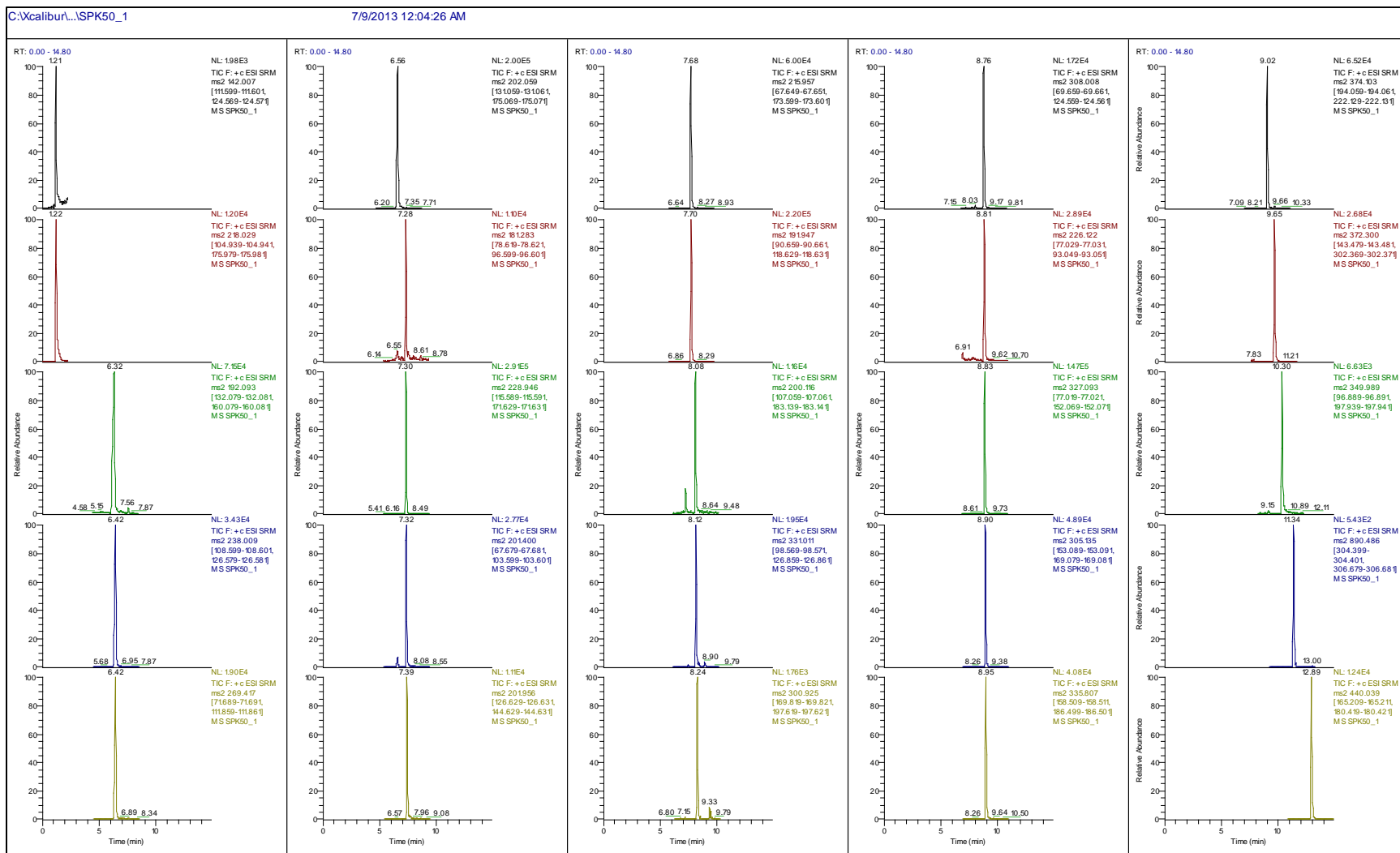
Name	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Methamidophos	1.21	142.007	124.57	14	111.6	5	60
Pymetrozine	1.22	218.029	104.94	18	175.98	16	70
Carbendazim	6.32	192.093	160.08	17	132.08	29	81
Dicrotophos	6.42	238.009	126.58	17	108.60	33	73
Acetachlor	6.42	269.417	111.86	15	71.69	33	72
Thiabendazole	6.56	202.059	175.07	24	131.06	31	103
DIMP	7.28	181.283	96.60	13	78.62	32	44
Tebuthiuron	7.30	228.946	171.63	17	115.59	26	72
Simazine	7.32	201.400	67.68	33	103.60	24	85
Carbaryl	7.39	201.956	144.63	7	126.63	30	40
Atrazine	7.68	215.957	173.60	16	67.65	35	79
DEET	7.70	191.947	118.63	15	90.66	28	92
Pyrimethanil	8.08	200.116	107.06	23	183.14	22	66
Malathion	8.12	331.011	126.86	12	98.57	23	60
Bifenazate	8.24	300.925	169.82	15	197.62	5	51
Tebuconazole	8.76	308.008	69.66	29	124.56	35	97
Cyprodinil	8.81	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	11.34	890.486	304.40	18	306.68	15	102
Bifenthrin	12.89	440.039	180.42	11	165.21	39	66

Results:

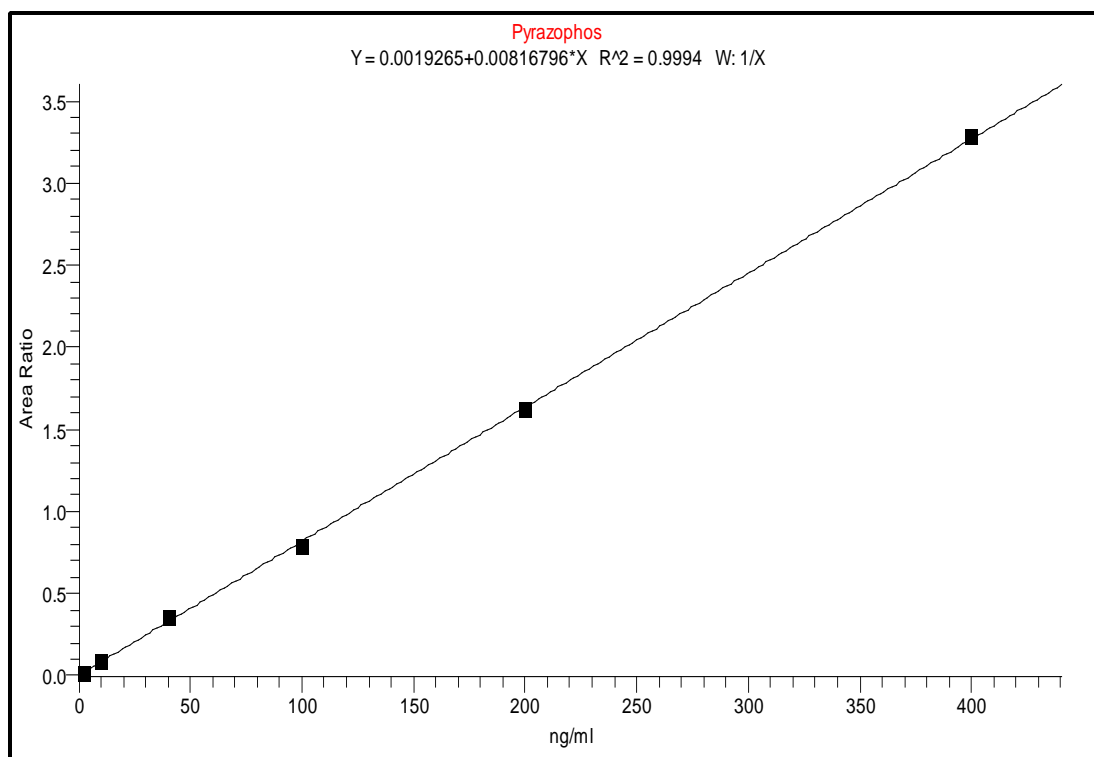
Recovery and RSD% Data Obtained from Fortified Banana Samples

Analytes	Spiked at 10 ng/g		Spiked at 50 ng/g	
	Recovery%	RSD% (n=5)	Recovery%	RSD% (n=5)
Methamidophos	97.3	5.9	100.2	4.6
Pymetrozine	96.5	4.7	99.3	3.8
Carbendazim	103.5	3.3	107.3	5.3
Diclotophos	101.8	4.1	104.8	4.8
Acetachlor	121.0	2.8	126.2	4.5
Thiabendazole	133.8	5.8	111.0	4.9
DIMP	89.2	6.0	92.1	7.7
Tebuthiuron	105.2	7.9	112.2	5.1
Simazine	96.3	4.6	101.2	4.8
Carbaryl	93.3	10.8	96.4	7.1
Atrazine	97.6	12.8	101.5	7.1
DEET	86.9	12.8	93.6	7.3
Pyrimethanil	100.6	8.0	97.0	5.7
Malathion	103.9	2.6	100.2	4.8
Bifenazate	84.4	13.7	85.4	3.2
Tebuconazole	90.0	1.2	88.2	1.5
Cyprodinil	97.3	3.1	96.0	1.8
Diazinon	104.1	1.7	99.8	2.9
Zoxamide	104.3	2.7	98.9	4.4
Pyrazophos	105.4	3.3	106.1	5.2
Profenofos	95.8	8.8	96.4	8.7
Chlorpyrifos	86.8	14.3	90.7	12.3
Abamectin	81.7	7.8	80.6	16.3
Bifenthrin	90.9	2.6	88.4	7.8
Mean	98.7	6.3	98.9	5.9

Chromatograms of a Fortified Banana Sample (50 ng/g)



Matrix Matched Calibration Curves of Pyrazophos ($R^2=0.9994$)



DCN-318170-269