Determination of Pesticides in Coffee with QuEChERS Extraction and Silica Gel SPE Cleanup

UCT Part Numbers

ECMSSC50CT-MP
50-mL centrifuge tube and Mylar pouch containing 4000 mg $MgSO_4$ and 1000 mg NaCl

CUSIL156
Clean-Up® silica gel
500mg/6mL column

GCLGN4MM-5
GC liner, 4mm splitless gooseneck
4mm ID x 6.5mm OD x 78.5mm

Summary:

Coffee is one of the most widely consumed beverages in the world, partly due to the stimulating effect of its caffeine content. Like most crops, the application of pesticides in coffee cultivation is a common practice in order to increase production yields. To ensure food safety it is important to test pesticide residues in coffee. However, analysis of pesticides in coffee is challenging because it contains a large amount of caffeine as well as acidic and polyphenolic matrix components that are typically co-extracted with the analytes of interest. These matrix components are difficult to remove during sample extraction and cleanup which can cause complications during instrumental analysis. Caffeine, in particular, can significantly compromise GC analysis.

QuEChERS is a well-established method for extraction of pesticide residues in fruit and vegetables, but dispersive-SPE cleanup is not adequate for coffee cleanup as large amounts of caffeine remain in the final extract. To overcome some of the limitations of existing methods there is a need to develop a sample preparation procedure that minimizes matrix effects while reducing the amount of caffeine in the final sample extract. This application details an optimized method for the extraction and cleanup of pesticide residues from coffee using a QuEChERS extraction procedure followed by a silica gel SPE cleanup. Twenty representative pesticides, most of which are commonly used pesticides on coffee farms [1], were evaluated in this study. GC-MS was used for pesticide detection and quantification.

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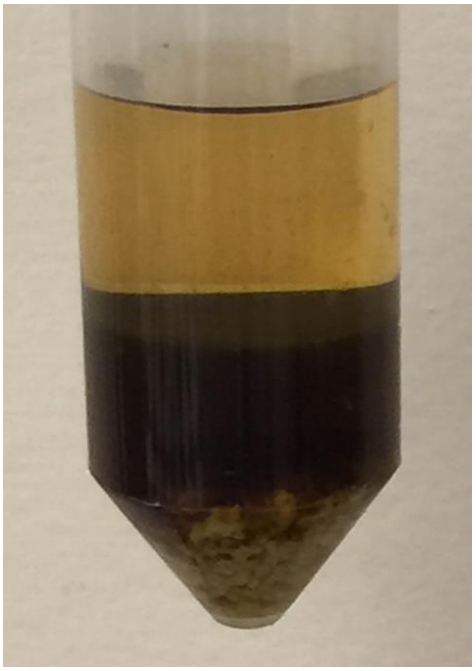
QuEChERS Procedure:

Sample Extraction:

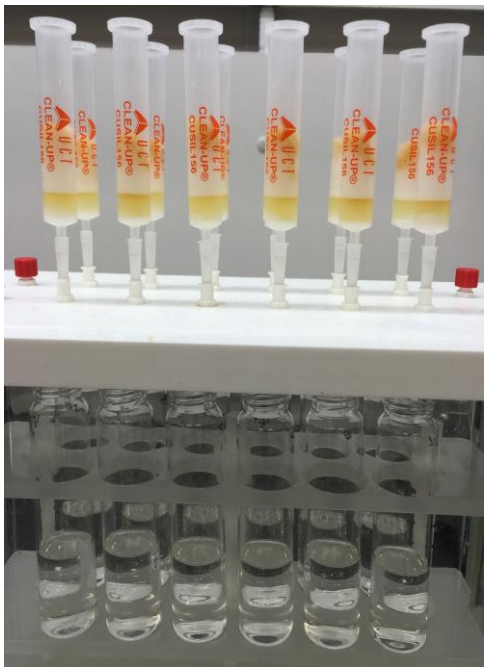
1. Add 10 mL brewed coffee (pH adjusted to about 8 with 1 N NaOH) and 10 mL acetonitrile (MeCN) to a 50-mL centrifuge tube.
2. Add the QuEChERS extraction salts from the Mylar pouch (ECMSSC50CT-MP) to the 50-mL tube, and shake vigorously for 1 min manually or using a Spex 2010 Geno-Grinder at 1000 strokes/min.
3. Centrifuge at ≥3000 rcf for 5 minutes
4. Transfer 5 mL supernatant to a clean test tube, add 1.5 mL toluene, and evaporate to about 1 mL.

Sample Clean-Up:

1. Add about ½ inch of anhydrous sodium sulfate to a silica gel SPE cartridge (CUSIL156), and attach the SPE cartridge to a glass block or positive pressure manifold.
2. Wash the SPE cartridge with 6 mL dichloromethane, soak for 1 min, drain to waste, and dry the SPE cartridge for 1 min under full vacuum or pressure.
3. Condition the SPE cartridge with 2 x 6 mL n-hexane by gravity.
4. Insert glass collection container into the manifold, load the 1 mL concentrated sample onto the SPE cartridge, rinse the test tube with 6 mL of 15% acetone in n-hexane and apply the rinsate to the SPE cartridge, and collect.
5. Continue to elute with 3 x 6 mL of 15% acetone in n-hexane by gravity.
6. Add 1.5 mL ethyl acetate to the eluate container and evaporate to 1 mL.
7. Add internal standard, vortex for 30 seconds, and inject 1 µL into the GC-MS for analysis.



QuEChERS extraction



Silica gel SPE cleanup

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GC-MS Parameters:

GC-MS Conditions	
Instrumentation	Agilent 6890N GC coupled to a 5975C MSD
Column	Restek Rxi®-5Sil MS (30m × 0.25mm × 0.25µm)
Carrier gas	Helium (1.2 mL/min)
GC inlet temp.	250°C
Injection volume	1 µL (splitless)
Temp gradient	60°C for 1 min, 10°C/min to 310°C, hold for 2 min; 28 min total
Transfer line temp	280°C
Ion source temp	250°C
Ionization mode	EI (70 eV)
Acquisition mode	Selective ion monitoring (SIM)

Compound Name	RT (min)	SIM Ions (25 ms dwell time)			R^2
TPP (IS)	21.625	326	325	77	NA
Carbaryl	12.630	144	115	116	0.9992
Tebuthiuron	12.725	156	171	74	0.9991
DEET	13.389	119	190	91	0.9977
Simazine	15.320	201	186	173	0.9989
Atrazine	15.400	200	215	173	0.9992
Diazinon	15.819	137	179	304	0.9986
Pyrimethanil	15.927	198	199	77	0.9980
Disulfoton	16.050	88	89	97	0.9986
Acetochlor	16.798	146	162	223	0.9975
Methyl parathion	16.935	109	125	263	0.9998
Malathion	17.618	125	173	93	0.9987
Chlorpyrifos	17.787	197	97	314	0.9983
Triadimefon	17.990	57	208	181	0.9982
Cyprodinil	18.456	224	225	210	0.9975
Endosulfan I	19.397	241	195	339	0.9984
Flutriafol	19.426	123	219	164	0.9970
Endosulfan II	20.518	195	241	339	0.9986
Tebuconazole	21.559	125	250	83	0.9999
Pyrazophos	23.362	221	232	373	0.9987
Cypermethrin (sum of 4 isomers)	25.000	163	181	209	0.9996

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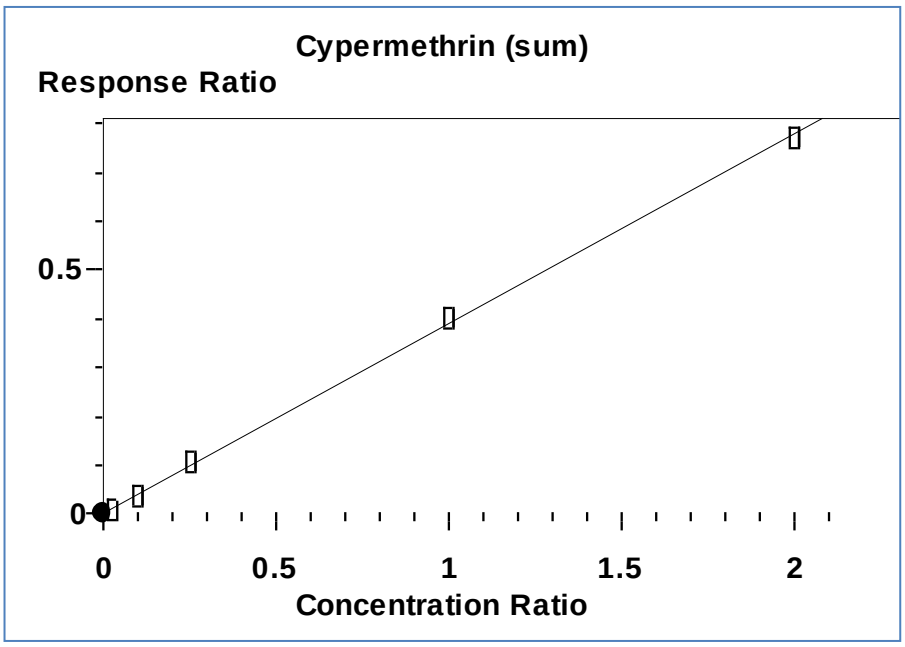
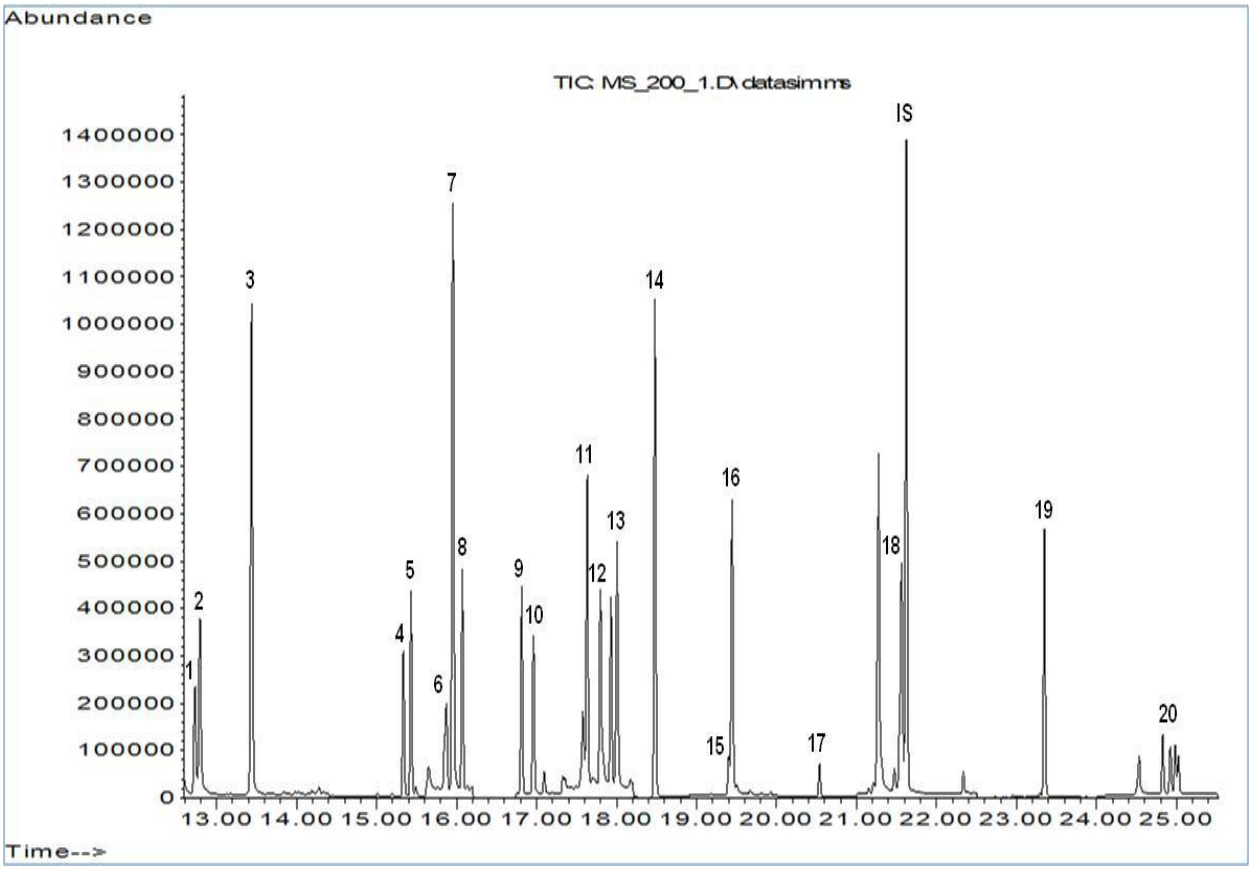
Results:



Matrix matched calibration curve of cypermethrin (5 - 400 ng/mL)



SIM chromatogram of an extracted coffee sample fortified with 200 ng/mL pesticides.
Peaks: 1) carbaryl; 2) tebuthiuron; 3) DEET; 4) simazine; 5) atrazine; 6) diazinon; 7) pyrimethanil; 8) disulfoton; 9) acetochlor; 10) methyl parathion; 11) malathion; 12) chlorpyrifos; 13) triadimefon; 14) cyprodinil; 15) endosulfan I; 16) flutriafol; 17) endosulfan II; 18) tebuconazole; 19) pyrazophos; 20) cypermethrin (sum of 4 isomers).

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Recovery and RSD% from Spiked Coffee Samples				
Compound Name	**Spiked at 20 ng/mL**		**Spiked at 200 ng/mL**	
	Recovery%	**RSD% (n=5)**	**Recovery%**	**RSD% (n=5)**
Carbaryl	100.2	5.0	98.7	1.6
Tebuthiuron	95.3	6.3	99.9	2.4
DEET	102.4	5.3	99.1	2.5
Simazine	103.5	5.4	98.6	1.2
Atrazine	103.6	6.5	97.9	2.4
Diazinon	124.4	9.9	99.6	2.2
Pyrimethanil	106.4	6.3	101.6	1.2
Disulfoton	88.1	7.1	92.5	2.2
Acetochlor	103.3	5.6	98.7	1.6
Methyl parathion	91.3	6.3	97.9	1.9
Malathion	103.0	7.7	99.9	3.6
Chlorpyrifos	103.6	6.9	99.4	1.3
Triadimefon	109.3	5.1	101.5	1.6
Cyprodinil	106.4	6.8	102.4	1.0
Endosulfan I	114.0	6.2	98.2	1.7
Flutriafol	74.5	11.6	87.9	4.7
Endosulfan II	103.7	6.1	99.5	1.3
Tebuconazole	92.7	8.5	101.8	1.5
Pyrazophos	98.0	7.5	101.4	1.4
Cypermethrin (sum)	97.0	5.1	101.7	1.0

References:

[1] http://www.coffeehabitat.com/2006/12/pesticides_used_2/

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