DETERMINATION OF PESTICIDES IN RED WINE BY QuEChERS EXTRACTION, QUICK QuEChERS CLEANUP AND LC/MS/MS DETECTION

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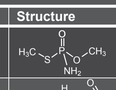
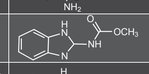
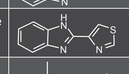
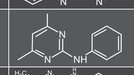
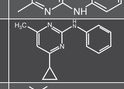
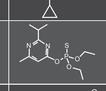
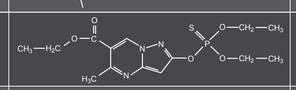
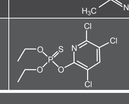
ENVIRO

INTRODUCTION

Red wine is a rich source of phenolic compounds, antioxidants that have heart-healthy and anticancer benefits [1]. The application of pesticides, such as fungicides and insecticides to improve grape yields, is common practice in vineyards. Pesticide residues may remain in the grapes after harvest and in the wines that are made from them. Therefore, it is important to analyze for the presence of pesticide residues in red wines. The analysis of pesticide residues in red wine is challenging due to the complexity of the matrix which contains alcohol, organic acids, sugars, phenols and pigments, such as anthocyanins. QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe) is a promising analytical approach that was first published in 2003 by Anastassiades et al. for the analysis of pesticide residues in vegetables and fruits [2]. The QuEChERS procedure involves the extraction of pesticides into acetonitrile (MeCN) with the aid of salts and buffers, followed by dispersive solid phase extraction (dSPE) to clean up co-extractives.

The aim of this study was to use a QuEChERS extraction, but develop a clean-up approach that is easier and faster than the dSPE used in QuEChERS. This sample clean-up method is based on the filter and clean concept: the red wine extract is passed through a push thru cartridge containing magnesium sulfate ($MgSO_4$) and primary and secondary amine (PSA). The co-extractives are retained onto the sorbents. The cleaned extract is collected in an auto-sampler vial and injected directly into LC/MS/MS for analysis. The clean-up procedure takes less than one minute per sample. Eight pesticides were selected for this study. Polarities of the pesticides were very different, with the logarithm of the octanol water partition coefficient (LogP) ranging from -0.779 to 5.004. Among the eight pesticides, Cyprodinil is the most common pesticide detected in grapes, while Chlorpyrifos, Diazinone, and Methamidophos are also frequently found in grapes [3].

Classes, structures, LogP and pKa values of the eight pesticides selected in this study

Compound	Class	Structure	LogP	pKa
Methamidophos	insecticide		-0.779	-0.58
Carbendazim	fungicide		1.52	5.66, 11.62
Thiabendazole	fungicide and parasiticide		2.47	3.40, 10.53
Pyrimethanil	fungicide		2.558	4.41
Cyprodinil	fungicide		3.012	4.22
Diazinone	insecticide		3.766	1.21
Pyrazophos	fungicide and insecticide		2.810	-1.37
Chlorpyrifos	insecticide		5.004	-5.28

EXPERIMENTAL

Materials

50 mL polypropylene centrifuge tube (UCT cat#: RFV0050CT)

Mylar Pouch containing 4000 mg $MgSO_4$ and 2000 mg NaCl (UCT cat#: ECQUUS2-MP)

Quick QuEChERS mini-cartridge containing 110 mg $MgSO_4$ and 180 mg PSA (UCT cat#: ECPURMPSMC)

Procedures

QuEChERS extraction

a. Add 10 mL red wine to 50 mL polypropylene centrifuge tubes.
b. Spike with appropriate amounts of target analytes for fortified samples, vortex 30 sec and equilibrate for 15 min.
c. Add 10 mL MeCN, vortex 30 sec.
d. Add salts in Mylar pouch ($MgSO_4$ and NaCl), shake vigorously for 1 min.
e. Centrifuge at 5000 rpm for 5 min, the supernatant is ready for cleanup.

Quick QuEChERS cleanup

a. Load 1 mL red wine extract with polypropylene syringe.
b. Pass the extract slowly through Quick QuEChERS mini-cartridge ($MgSO_4$ and PSA).
c. Collect 0.5 mL cleaned extract into 2 mL auto-sampler vial.
d. Add 10 µL 5 ppm triphenyl phosphate as internal standard, the extract is ready for LC/MS/MS analysis.

Photos showing the cleanup procedure:

a. Load 1 mL red wine extract, attach to Quick QuEChERS mini-cartridge

b. Pass red wine extract through the mini-cartridge slowly, collect 0.5 mL cleaned extract

Comparison of mini-cartridges before (left) and after (right) cleanup of 1 mL red wine extract

Red wine extract

Red wine extract after Quick QuEChERS cleanup

INSTRUMENTAL

LC: Thermo Accela 1250 pump with PAL auto-sampler

LC Conditions

Column	Guard column: Restek C18, 2.1 x 20 mm Column: Sepax HP-C18, 2.1 x 100 mm, 3 µm, 120 Å
Column Temperature	Ambient
Injection Volume	10 µL at 15° C
Mobile Phase	A: 0.1% formic acid in Milli-Q-water B: 0.1% formic acid in LC/MS grade methanol
Flow Rate	200 µL/min

LC Gradient Program

Time	%A	%B
0	95	5
1	95	5
3	50	50
8	5	95
14	5	95
14.2	95	5
16	95	5

MS/MS: Thermo TSQ Vantage tandem MS

MS Conditions

Ion source:	Heated ESI
Ion polarity:	Positive
Spray voltage:	3000 V
Sheath gas pressure:	N_2 @ 40 psi
Auxiliar gas pressure:	N_2 @ 10 psi
Ion transfer capillary temperature:	350C
Scan type:	SRM (0-16 min.)
CID conditions:	Ar @ 1.5 mTorr

SRM transitions

Compound	Parent ion	Product ion 1	CE	Product ion 2	CE	S-Lens	Dwell time (s)
Methamidophos	142.044	94.090	14	125.050	16	59	0.15
Carbendazim	192.093	132.080	29	160.080	17	81	0.10
Thiabendazole	202.059	131.060	31	175.070	31	103	0.10
Pyrimethanil	200.116	107.060	23	183.140	22	66	0.10
Cyprodinil	226.122	77.030	40	93.050	33	88	0.10
TPP (IS)	327.093	77.020	37	152.070	33	98	0.10
Diazinone	305.135	153.090	15	169.08	14	89	0.10
Pyrazophos	374.103	194.060	20	222.130	20	104	0.10
Chlorpyrifos	349.989	96.890	32	197.940	17	69	0.10

RESULTS AND DISCUSSIONS

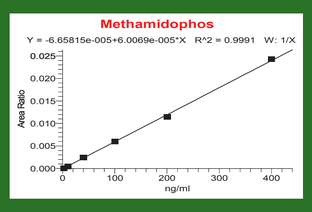
Matrix matched calibration, limit of detection (LOD), and limit of quantification (LOQ)

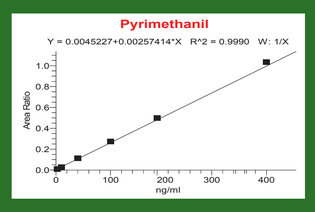
Calibration curves were obtained by analysis of matrix matched standards, which were prepared by spiking appropriate amounts of 2 ppm pesticide mixture to blank red wine extracts after Quick QuEChERS cleanup. Six matrix matched standards at 2, 10, 40, 100, 200, and 400 ng/mL levels were analyzed. The responses were linear over the calibration range. LOD and LOQ are the concentrations that give signal-to-noise ratio (S/N) of 3 and 10 respectively. In this study they were estimated according to the S/N values of the lowest matrix matched calibration level of 2 ng/mL.

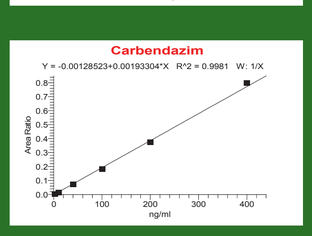
Matrix matched calibration, LOD and LOQ

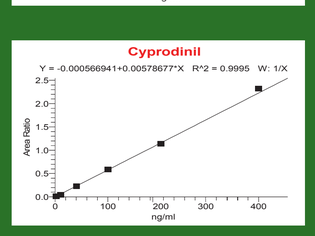
Compound	Linearity range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Methamidophos	2-400	0.9991	0.15	0.49
Carbendazim	2-400	0.9981	0.40	1.33
Thiabendazole	2-400	0.9940	0.09	0.31
Pyrimethanil	2-400	0.9990	0.01	0.05
Cyprodinil	2-400	0.9995	0.17	0.57
Diazinone	2-400	0.9982	0.06	0.21
Pyrazophos	2-400	0.9976	0.08	0.27
Chlorpyrifos	2-400	0.9981	0.10	0.32

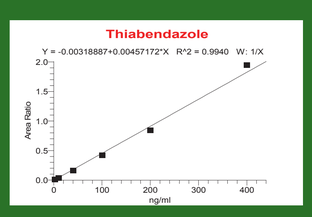
Calibration Curves

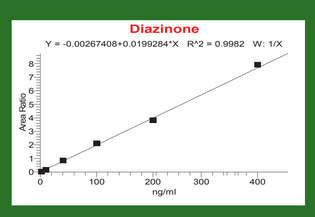


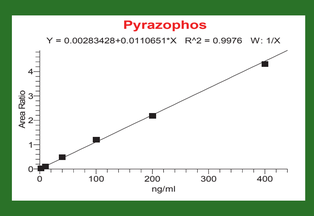


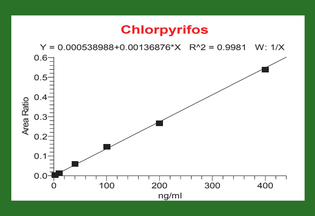












ACCURACY AND PRECISION DATA

Red wine samples fortified with 10, 50, and 100 ng/mL target pesticides were extracted with QuEChERS and cleaned up with Quick QuEChERS mini-cartridges. Recoveries ranged from 81.6% to 112.2% with overall recovery of 97.0%. Relative standard deviations (RSD) based on four replicates for three spiking levels were less than 10.8%. The recovery and RSD data indicated that this method is accurate and precise for the determination of pesticide residues in red wine samples.

Graphitized carbon black (GCB), which is often used to clean up pigments in vegetables and other food samples, is known to retain planar compounds, such as Carbendazim, Thiabendazole, Pyrimethanil and Cyprodinil, resulting in low recoveries of those pesticides. In this study PSA instead of GCB was used to clean up red wine pigments. The recoveries of the four planar pesticides included in this study were not affected (≥85%).

Accuracy and Precision Data

Compound	Fortified at 10 ng/mL		Fortified at 50 ng/mL		Fortified at 100 ng/mL	
	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)
Methamidophos	93.7	3.4	81.6	5.8	84.2	3.5
Carbendazim	105.7	10.8	100.1	10.6	90.5	7.6
Thiabendazole	91.2	4.9	87.9	6.8	85.0	4.0
Pyrimethanil	112.2	2.7	107.0	3.2	102.8	4.9
Cyprodinil	104.3	3.2	99.9	6.1	100.2	4.9
Diazinone	104.9	104.9	96.6	6.6	99.2	6.8
Pyrazophos	99.9	4.0	96.6	5.6	91.3	4.1
Chlorpyrifos	91.7	4.6	99.5	5.2	97.2	3.8

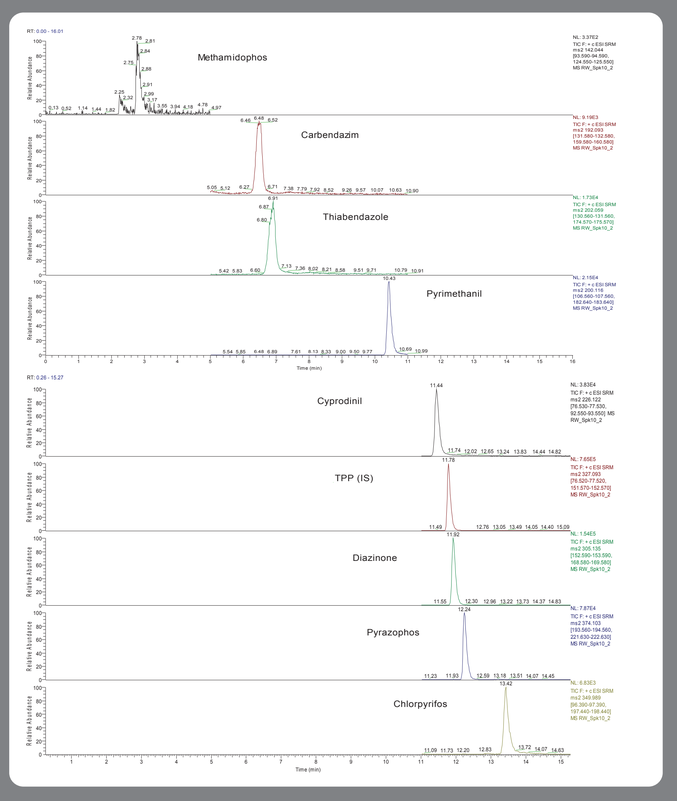
APPLICATION TO REAL SAMPLES

Six red wine samples were tested using this simple, fast, and novel method. Carbendazim was detected at 10.2, 8.7, and 2.3 ng/mL in red wine sample 4, 5, and 6 respectively. The detected concentrations are much lower than the EU or Japan regulated levels (ppm) in grapes.

Pesticides detected in red wine samples (ng/mL)

Compound	Sample 1 (Santa Carolina)	Sample 2 (Liberty Creek)	Sample 3 (Rossi)	Sample 4 (Great Wall)	Sample 5 (Rosemount)	Sample 6 (Two Hands)
Methamidophos	< 2	< 2	< 2	< 2	< 2	< 2
Carbendazim	< 2	< 2	< 2	**10.2**	**8.7**	**2.3**
Thiabendazole	< 2	< 2	< 2	< 2	< 2	< 2
Pyrimethanil	< 2	< 2	< 2	< 2	< 2	< 2
Cyprodinil	< 2	< 2	< 2	< 2	< 2	< 2
Diazinone	< 2	< 2	< 2	< 2	< 2	< 2
Pyrazophos	< 2	< 2	< 2	< 2	< 2	< 2
Chlorpyrifos	< 2	< 2	< 2	< 2	< 2	< 2

Chromatograms of red wine sample 1 fortified with 10 ng/mL pesticides



CONCLUSIONS

An easy, fast, novel, and efficient clean-up method for red wine samples was successfully developed in this study. Pesticide residues in red wine samples were extracted into acetonitrile using QuEChERS. Clean-up was accomplished by passing 1 mL of red wine extract through a push thru cartridge containing $MgSO_4$ and PSA. $MgSO_4$ adsorbed water remaining in the extract, while PSA removed organic acids, sugars, and pigments. This clean-up method, based on the filter and clean concept, takes less than one minute per sample. Combined with the QuEChERS extraction, this method is an excellent choice for high throughput laboratories.

References:
[1] http://www.mayoclinic.com/health/red-wine/HB00089
[2] M. Anastassiades, S.J. Lehotay, D. Stajnbaher and F.J. Schenck, J. AOAC Int. 86(2), 412-431 (2003)
[3] http://www.whatsonmyfood.org/food.jsp?food=GR

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