Optimizing Recoveries of Planar Pesticides in Spinach using Toluene and the AOAC QuEChERS Kits with Graphitized Carbon

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RAFA

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Introduction

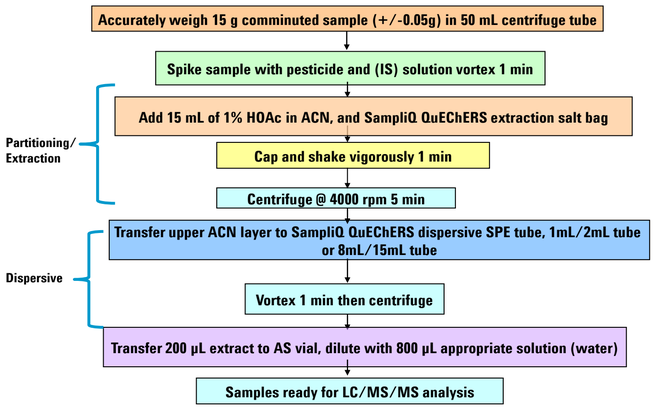
- Regulatory agencies have stated that spinach is one of a dozen most heavily pesticide contaminated produce products
- QuEChERS (**Qu**ick **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe) methodology has been employed to extract and quantitate pesticides in
- Spinach is a dark green plant: Graphitized Carbon Black (GCB) is required in the dispersive SPE kits in order to remove high levels of pigments from the spinach matrix
- GCB not only removes pigments like chlorophyll, it also retains pesticides with planar structures resulting in poor recovery and precision
- In order to compensate for GCB negative effects on planar pesticide recoveries we evaluated the impact of Toluene addition in the dispersive SPE step to the analysis of pesticides in spinach using SampliQ QuEChERS AOAC kits for highly pigmented fruits and vegetables with analysis via LC/MS/MS

Spinach really does make you strong like popeye, scientists say

www.dailymail.co.uk/health/article-1018637/eating-spinach-really-CAN-Popeyes-strength.html

Experimental

QuEChERS: General Approach



Representative Pesticides used in the Study: LC/MS/MS

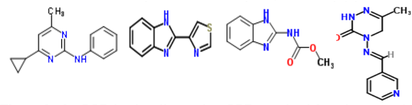
Compound	Category	Log P	LC/MS/MS MRM channel
Acephate	Organophosphate	-0.89	184>143
Carbaryl	Carbamate	2.36	202>145
Carbendazim *	Benzimidazole	1.48	192>160
Cyprodinil *	Anilnopyrimidine	4	225.9>108
Dichlofluanid	Sulphamide	3.7	332.7>223.8
Imidacloprid	Neonicotinid	0.57	255.9>209
Methamidophos	Organophosphate	-0.82	141.8>112
Penconazole	Triazole	3.72	283.8>159
Propoxur	Carbamate	0.14	210>111
Pymetrozine *	Pyridine	-0.93	217.9>105
Thiabendazole *	Benzimidazole	2.39	201.8>174.9
Thiophanate-methyl	Benzimidazole	1.16	342.8>151
Tolylfluanid	Sulphamide	3.9	346.7>237.9
Ethoprophos	Organophosphate	2.99	242.8>173
Kresoxim-methyl	Strobilurin	3.4	314>267
TPP (IS)	Organophosphate	4.10	327.1>77

Table 1: Compounds identified by asterisks (*) are the problematic planar pesticides evaluated in this study

Experimental

Preliminary Evaluation of Pesticide Recovery

- **The Problem**: the planar pesticides: pymetrozine, carbendazim, thiabendazole, cyprodinil recoveries and precision were poor, noted by (*) in Table 1



- **The culprit:** GCB in the dispersive-SPE required for pigmented produce, not only does GCB remove pigments like chlorophyll; it also retains planar pesticides
- **Recovery range:** for the planar pesticides was 20-40%, poor but not unusual, however the precision was unbelievably poor, which is **NOT** the normal expected precision > 15% RSD
- **The solution:** addition of Toluene, which has been shown in other labs to substantially improve the recoveries and precision of planar pesticides from highly pigmented produce

Results and Discussion

Optimizing Toluene Addition

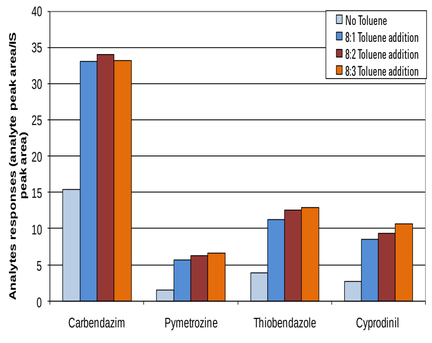


Figure 1: Results comparison of different Toluene addition volume. First column: results generated with no Toluene addition; second column: results generated with Toluene addition at ratio of 8:1 (ACN extracts/Toluene); third column: results generated with Toluene addition at ratio of 8:2; fourth column: results generated with Toluene addition at ratio of 8:3.

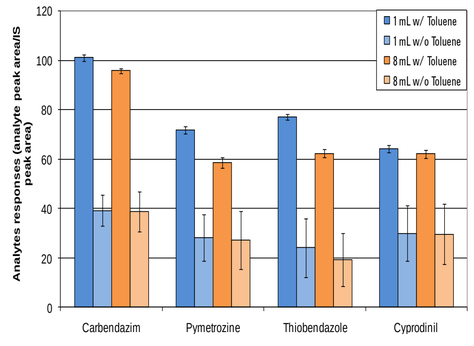
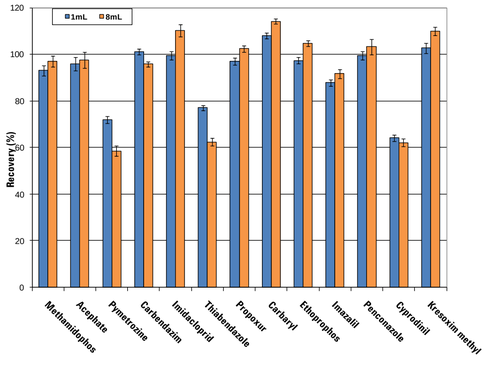
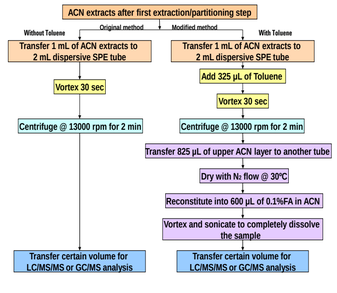


Figure 2: Results comparison of 1mL and 8mL dispersive SPE with the modified method (w/ Toluene) and the original method (w/o Toluene).

Exceptional Recoveries and Precision for 1 mL and 8 mL Dispersive-SPE



Modified QuEChERS Approach for Highly Pigmented Fruits and Vegetables with and without Toluene



Results and Discussion

Comparison of LC/MS/MS Chromatograms Representing Improved Recovery for Planar Pesticides with Toluene Addition

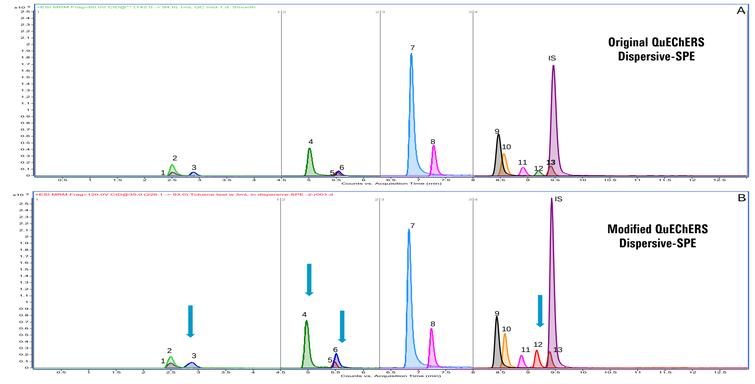


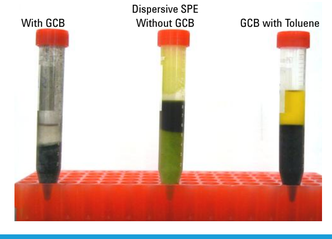
Figure 3: LC/MS/MS chromatograms of 50 ng/g fortified spinach sample extracts processed by original dispersive SPE (A) and modified dispersive SPE (B). Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine*, 4. Carbendazim*, 5. Imidacloprid 6. Thiabendazole*, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos, 10. Imazalil, 11. Penconazole, 12. Cyprodinil*, 13. Kresoxim methyl IS: Internal Standard, TPP. Vertical arrows indicate enhanced pesticide recovery after Toluene addition for the planar pesticides (*)

Positive Effects of Toluene Addition with Dispersive-SPE Containing GCB

Analytes	Original method (w/o toluene) Recovery	RSD (n=6)	Modified method (w/ toluene) Recovery	RSD (n=6)	Impact with modified method	Detection method
Carbendazim	38.9	14.6	98.5	2.5	Positive	LC/MS/MS
Thiabendazole	21.8	19.7	69.7	2.7	Positive	LC/MS/MS
Pymetrozine	27.6	21.2	65.2	3.7	Positive	LC/MS/MS
Cyprodinil	29.6	23.4	63.1	3.2	Positive	LC/MS/MS

No adverse effects were seen with the addition of Toluene on the representative pesticides evaluated

Visual Observation with Toluene Addition to Dispersive-SPE



Conclusions

- Agilent's SampliQ QuEChERS AOAC buffered extraction kit and dispersive SPE kits for pigmented fruits and vegetables provide a simple, fast, and effective method for purification of representative pesticides in spinach
- **Modified dispersive SPE with the addition of Toluene** provides a very useful way to limit the loss of planar pesticides caused by the amount of GCB in the dispersive SPE kit (50 or 400 mg AOAC dispersive kit for pigmented fruits and vegetables)
- **The 8:3 ratio of ACN:Toluene is similar to the ratio defined by Frank Schenck**, "*Determination of Pesticides in Food of Vegetal Origin, Analysis of Pesticides in Food & Environmental Samples", Chapter 6, CRC Press, 2008*
- Impurities and matrix effects from the spinach with the addition of Toluene were minimal and did not interfere with quantitation of the pesticides
- **Dramatic increase in recovery 50-300% (91.9% on average) and reduced RSDs 66% (3.3% on average)** directly attributed to the addition of Toluene to the dispersive SPE kits
- **Limit of quantitation (LOQ) 5 ng/g was well below the maximum residue limit (MRLs)**