Optimizing Sample Preparation for the Analysis of over 200 Multi-Residue Pesticides in Produce by GC-MS/MS

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Abstract

Optimization of sample preparation is an important step in mitigating matrix effects in the multi-residue pesticides workflow. Using the QuEChERS approach followed by dispersive solid phase extraction (dSPE) allows for customization of solutions based on matrices. In order to efficiently select the extraction and clean-up combination that yields the highest analyte response while providing sufficient matrix removal, we first tested 40 representative pesticides with different QuEChERS extraction salts and dSPE clean-up sorbents. The results were evaluated based on: the responses of all tested pesticides, response of commodity relevant pesticides, and the overall cleanliness of the samples. To demonstrate the feasibility of developing optimized methods, organic celery and other representative matrices were spiked with over 200 pesticides at two levels, 100 ppb and 10 ppb. Non-spiked commodities were also analyzed for the presence of incurred pesticides. Chromatogram separations were performed using a Rxi-5MS column (30 m x 0.25 mm x 0.25 µm) and analyzed by GC-MS/MS in selected reaction monitoring mode. Optimized sample preparation conditions, pesticide separations, and recovery and incurred pesticide results from organic celery, spinach, and orange are presented.

Available dSPE cleanup

Cat #	Material	Vol	Sample Type
26124	150 mg MgSO4, 50 mg PSA	2 mL	General fruits and vegetables
26215	150 mg MgSO4, 25 mg PSA	2 mL	General fruits and vegetables
26216	150 mg MgSO4, 25 mg PSA, 25 mg C18-EC	2 mL	Foodstuffs with fats and waxes
26242	150 mg MgSO4, 50 mg C18-EC	2 mL	Foodstuffs with fats and waxes
26125	150 mg MgSO4, 50 mg PSA, 50 mg C18-EC	2 mL	Foodstuffs with fats and waxes
26217	150 mg MgSO4, 25 mg PSA, 2.5 mg GCB	2 mL	Pigmented fruits and vegetables
26123	150 mg MgSO4, 50 mg PSA, 50 mg GCB	2 mL	Pigmented fruits and vegetables
26218	150 mg MgSO4, 25 mg PSA, 7.5 mg GCB	2 mL	Highly pigmented fruits and vegetables
26219	150 mg MgSO4, 50 mg PSA, 50 mg C18-EC, 50 mg GCB	2 mL	Highly pigmented fruits and vegetables
26243	150 mg MgSO4, 50 mg PSA, 50 mg C18-EC, 7.5 mg GCB	2 mL	General purpose (wide variety of sample

Optimized QuEChERS salts and dSPE selection

Matrix	Salts	dSPE	PSA	C18-EC	GCB
Celery	AOAC	26215	25	--	0
Spinach	AOAC	26219	50	50	50
Orange (Pulp)	AOAC	26124	50	--	--
Orange (peel)	EN	26216	25	25	--
Orange (whole)	EN	26125	50	50	--
Avocado	Original Unbuffered	26125	50	50	--
Brown Rice Flour	Original Unbuffered	26125	50	50	--
Honey	AOAC	26124	50	--	--

Low Moisture Adjustments

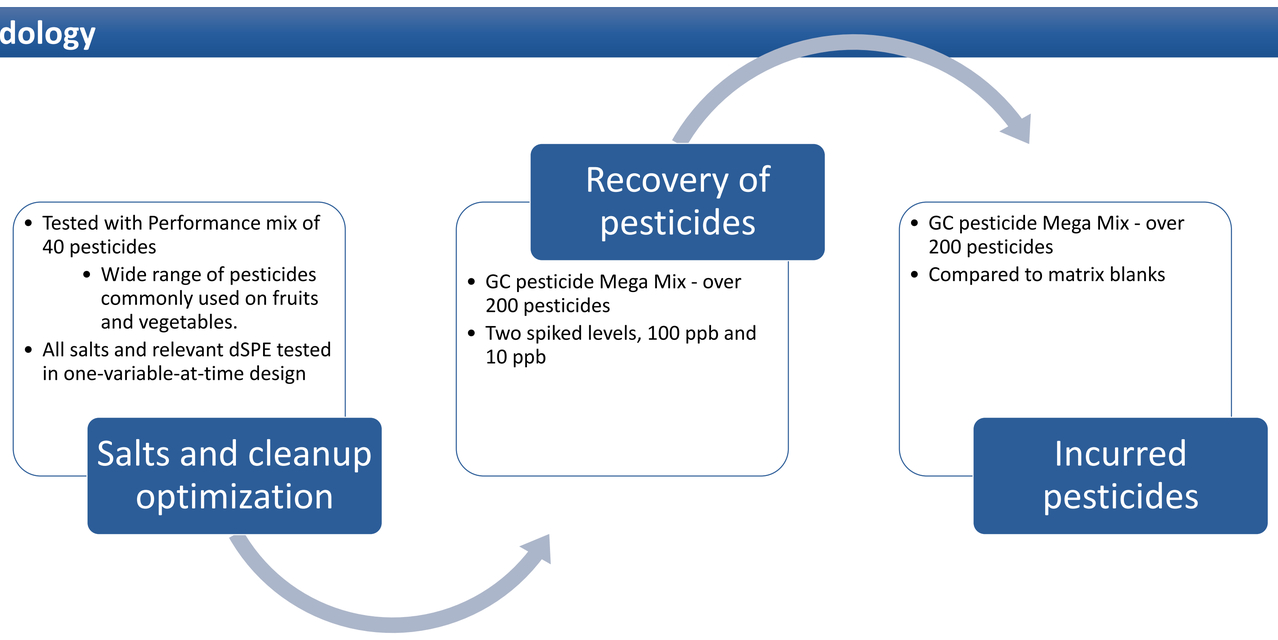
Water addition

- Water is essential to help the solvent (ACN) to reach all parts of the matrix
- Generally there needs to be a same amount of water as solvent (ACN)

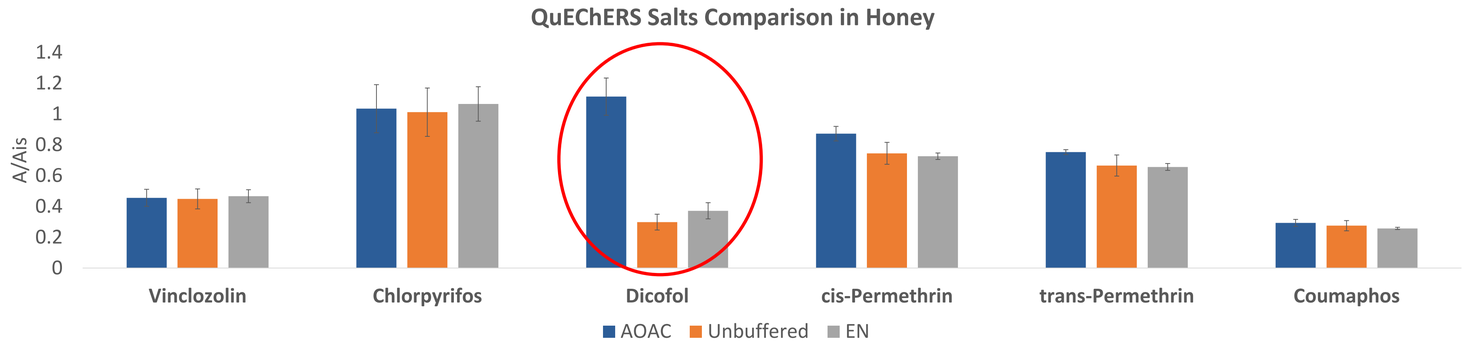
Reducing matrix loading

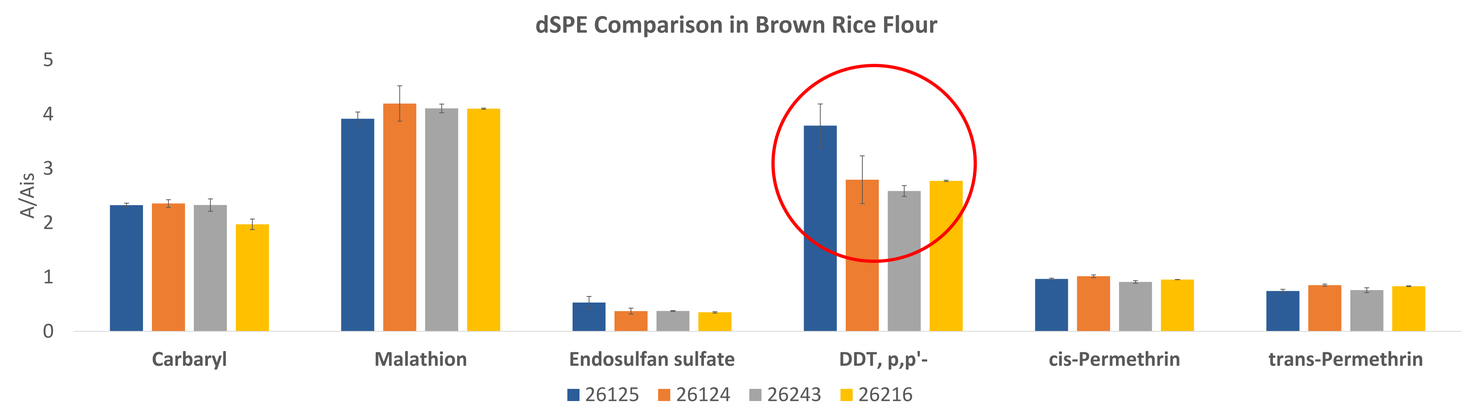
- Reducing the amount of matrix is beneficial when the commodity is dense and/or hard to hydrate because it helps with water/solvent penetration of matrix

Methodology



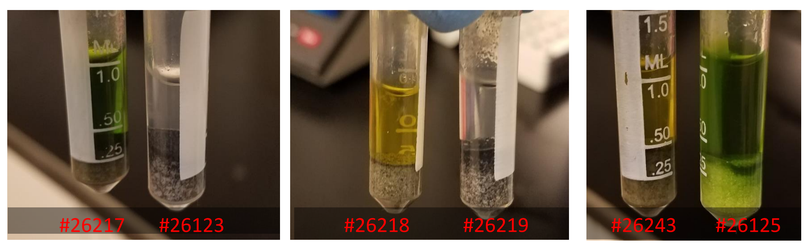
Optimization Step – Examples





Optimization Step – Removal of Pigments

Comparison of dSPE cleanup was made using both recoveries and visual comparison of pigment removal. While there was a clear visual difference, the recoveries were very similar. To achieve a efficient cleanup, high graphitized carbon dSPE has to be used.



Optimization Step – How to Approach Orange?

Why separate peel and pulp?

- Pulp is the edible part
  - High content of water and sugars => High PSA content in the cleanup is needed
- Peel is used as source of orange flavor and essential oil as well as source of orange zest
  - High content of terpenes (essential oils), lower level of water and sugars => Low level of end-capped C18 (C18-EC) helps with efficient cleanup

Why analyze whole orange?

- Relevance to products that consist of both, such as orange juice
- Relatively high level of sugar from the pulp together with the essential oils from the peel => Higher PSA together with C18-EC is needed for cleanup
- While the total water content is around 80%, there is a benefit to adding water with commodity for the QuEChERS to help hydrate the peel

Pesticides' Recoveries

%	Celery	Spinach	Orange pulp	Orange peel	Orange whole	Avocado	Brown Rice Flour	Honey
150+	0	0	0	0	0	0	2	0
150–120	0	2	1	0	1	4	5	1
120–70	202	197	194	199	198	183	172	201
70–50	0	1	3	0	0	9	14	1
50–20	0	0	0	0	1	2	1	0
20–1	0	0	1	0	1	0	0	0
ND	1	3	4	4	2	5	9	0

Incurred Pesticides

- **Celery**
  - Cypermethrin 2.6 ± 0.3 ppb
  - Flutriafol 3.1 ± 0.7 ppb
  - Malathion 6.8 ± 0.4 ppb
- **Spinach**
  - Metalaxyl 3.4 ± 0.9 ppb
  - *trans*-Permethrin 2.0 ± 0.1 ppb
- **Orange pulp**
  - Fludioxonil 6.8 ± 0.2 ppb
- **Orange peel**
  - Fludioxonil 601 ± 27 ppb
  - Cypermethrin 8.8 ± 0.7 ppb
- **Orange (whole)**
  - Fludioxonil 322 ± 2 ppb
  - Cypermethrin 5.1 ± 0.4 ppb
- **Honey**
  - 3-4-Dichloroaniline 2.5 ± 1.0 ppb
  - 2-4-DPF 8.9 ± 0.3 ppb

Conclusions

- Sample prep method development is extremely important and evaluating multiple QuEChERS salts and cleanups is beneficial for higher recoveries
  - Splitting orange into peel and pulp can help with matrix effects and recoveries due to separating two very different matrices
- Recoveries were within 70 – 120% for 86 – 99.5% of the pesticide residues analyzed
- With exception of orange peel, no commodity studied had any incurred pesticide great than 9 ppb