A High-Throughput Approach to Multi-Matrix Food Testing Using QuEChERS and Tandem GC/MS

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Introduction

High throughput food laboratories using QuEChERS for multi-residue analysis in a variety of matrices face significant challenges since not all matrices will yield the same level of co-extractives. Nonvolatile co-extractives can contaminate the system and limit sample throughput. One approach is to replace dispersive SPE with a SPE cartridge containing an increased amount of an appropriate sorbent to increase capacity; however this requires a conditioning step, vacuum/positive pressure system, and evaporation of eluted volume - all of which are avoided by utilizing QuEChERS methodology. We have implemented a simple strategy when using a "just enough" sample purification technique such as QuEChERS in order that difficult samples may be handled in a cost-effective and efficient manner – without loss in recovery. This involves a second dispersive step in series in order to streamline multi-matrix sample preparation.

Experimental

Source of Methodology

The entire method, including sample preparation, is based on an external laboratory's production protocol. This currently calls for one d-SPE step and the ESTD method without the use of analyte protectants. In the study presented here, the ISTD anthracene-D10 was monitored and the use of protectants was evaluated.

Preparation of Winter Squash Extracts at 2xLOQ

15 g of homogenized winter squash (Robot Coupe; no dry ice) was placed into a 50 mL PP centrifuge tube.

Pre-extraction spiked samples:

Spike homogenized sample in the tube with 32xLOQ stock standard to yield a 2xLOQ/g final sample. Add anthracene-D10 to yield 200 ppb in the final extract. Mix.
Add 2 ceramic homogenizers, 15 mL acetonitrile, vortex 1 minute.
Add AOAC QuEChERS extraction salt packet (6 g $MgSO_4$, 1.5 g Na Acetate, PN 5982-6755), shake vigorously for 1 minute, centrifuge 4000 rpm, 5 minutes.

- For 1x d-SPE:

Transfer 9 mL of ACN extract to 15 mL dispersive-SPE tube (400 mg PSA and 1200 mg $MgSO_4$, PN 5982-5058), vortex 1 min, centrifuge 4000 rpm, 5 minutes.

Transfer to silanized GCMS vial PN 5183-2072 for analysis.
QS to 1.0 mL with 500 ppb each 3-ethoxy-1,2-propanediol and L-gulonic acid-γ-lactone (protectants).

Experimental cont.

- For 2x d-SPE:

Transfer 5 mL of ACN extract from the 1x d-SPE step to a 15 mL dispersive SPE tube (400 mg PSA and 1200 mg $MgSO_4$, PN 5982-5058), vortex 1 min, centrifuge 4000 rpm, 5 min.
Transfer to silanized GCMS vial for analysis. QS to 1.0 mL with 500 ppb each 3-ethoxy-1,2-propanediol and L-gulonic acid-γ-lactone (protectants).

Post-extraction spiked samples:

Spike blank extracted sample with stock standard to yield a 2xLOQ/g sample. QS to 1 mL with 200 ppb anthracene-D10 and 500 ppb each 3-ethoxy-1,2-propanediol and L-gulonic acid-γ-lactone.

GC-MS/MS Analysis by EI-MRM

Analysis was performed on an Agilent 7890A Gas Chromatograph coupled to a 7000B Triple Quadrupole Mass Spectrometer equipped with a Multi Mode Inlet and Purged Ultimate Union used for back flushing the column. The union was placed between two HP-5MSUI columns of dimensions 5m x 0.25mm x 0.25µm and 15m x 0.25mm x 0.25µm (PN 19091S-431UI). The inlet was programmed in cold splitless mode from 60 to 280°C at 725°C/min. and 1 µL was injected. A 2 mm dimpled liner (PN 5190-2296) was used. The oven was programmed to reach 310°C in constant flow mode. A two minute post-run back flush commenced at 26.6 min.

The MS source temperature was 310°C. An EM gain of 80 was used for this analysis. MRM transitions were distributed among nineteen time segments.

Recovery and Precision Experiments

Percent recovery was calculated by comparing analyte response obtained with pre-extraction spiked samples to those of post-extraction spikes for both 1x d-SPE and 2x d-SPE extracts:

$$\text{Peak Area}_{\text{Pre-spiked}} / \text{Peak Area}_{\text{Post-spiked}} \times 100$$

%RSDs were calculated for each of the four sample types using multiple injections performed on the same day: pre- and post-spiked 1x d-SPE extract and pre- and post-spiked 2x d-SPE extract (see table).

Recovery and same-day precision results presented in the table were obtained after over 100 injections were made on the GC columns. Over 740 injections of similar samples had been made on the MS source.

Comparison of techniques by inter-day consecutive injections was performed with new GC columns and liners for each set in order to better control these variables.

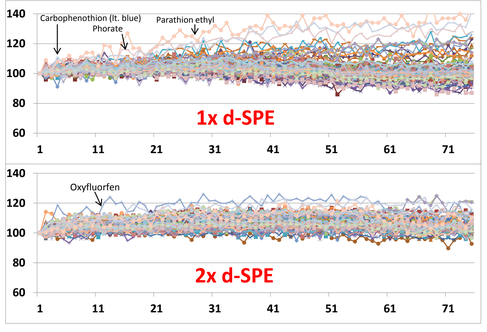
Recovery and Precision for One vs. Two QuEChERS d-SPE Steps

	RT	m/z	Conc. (ppb)	% Recovery 1x d-SPE	% Recovery 2x d-SPE	% RSDs 1x d-SPE Pre-spike (n=3)	% RSDs 1x d-SPE Post-Spike (n=8)	% RSDs 2x d-SPE Pre-spike (n=3)	% RSDs 2x d-SPE Post-Spike (n=8)	Δ %RSD 1x - 2x d-SPE Pre-spike	Δ %RSD 1x - 2x d-SPE Post-Spike
Dichlobenil	5.08	170.9 -> 136.0	20	88	89	0.4	1.2	0.4	0.4	0	0.7
Propham	6.95	179.1 -> 93.1	25	87	90	1.8	2.1	1.1	1.0	0.7	1.0
THPI	7.26	151.1 -> 80.1	60	84	84	1.1	0.8	1.2	2.8	-0.1	-2.0
1-Naphthol	7.72	144.1 -> 115.1	40	72	68	1.5	3.1	3.3	2.3	-1.8	0.7
PCB	7.99	249.8 -> 141.9	10	79	81	0.8	1.6	2.4	1.8	-1.6	-0.3
Diphenylamine	9.45	169.1 -> 167.1	20	83	84	0.9	1.2	0.7	1.7	0.2	-0.6
Chlorpropham	9.98	212.9 -> 127.1	30	87	91	0.8	1.7	1.1	1.7	-0.2	0
Ethalfluralin	10.20	276.0 -> 202.2	40	88	102	3.0	1.8	6.6	2.8	-3.6	-1.0
Trilfluralin	10.50	306.1 -> 264.1	30	88	104	3.4	1.5	6.8	2.5	-3.4	-1.1
Phorate	10.55	260.0 -> 75.0	20	89	92	1.2	2.6	1.8	1.9	-0.6	0.7
alpha-BHC	10.57	218.8 -> 183.0	10	87	89	0.8	1.2	1.1	2.7	-0.3	-1.5
HCB	10.75	283.8 -> 213.9	10	79	79	0.7	2.2	1.7	1.8	-1.0	0.4
Dicloran	10.90	205.9 -> 124.0	40	91	99	1.6	1.5	2.0	4.2	-0.4	-2.7
Clomazone	11.45	204.1 -> 107.2	15	88	89	1.2	1.1	1.3	1.1	-0.1	0
Lindane	11.50	218.8 -> 183.0	10	88	90	0.9	2.3	2.1	2.1	-1.1	0.2
PCNB	11.65	294.9 -> 236.8	20	86	93	1.6	2.8	3.4	1.6	-1.8	1.3
Terbufos	11.75	231.0 -> 129.0	10	90	96	1.7	1.7	2.7	1.5	-1.0	0.2
Anthracene-D10	11.80	188.0 -> 160.1	200	94	97	0.4	1.2	0.7	0.5	-0.3	0.6
Fonofos	11.82	246.0 -> 109.1	10	89	92	0.7	2.1	1.9	1.4	-1.1	0.7
Pronamide	11.96	172.9 -> 145.1	10	88	94	1.1	1.4	2.9	2.6	-1.8	-1.3
Terbacil	12.38	160.9 -> 144.1	50	88	92	1.3	2.4	3.5	1.4	-2.2	1.0
Triallate	12.50	267.9 -> 184.1	20	88	90	1.2	1.6	0.9	1.4	0.3	0.2
Tefluthrin	12.67	197.1 -> 141.0	20	89	93	1.1	2.4	2.3	1.6	-1.2	0.8
Chlorpyrifos Methyl	13.40	286.0 -> 270.9	50	88	96	2.8	1.1	3.9	2.9	-1.0	-1.9
Vinclozolin	13.45	284.9 -> 212.0	20	91	92	1.4	4.1	4.0	3.7	-2.6	0.4
Heptachlor	13.47	272.1 -> 236.9	20	85	90	2.1	1.4	3.2	1.7	-1.1	-0.3
Ametryn	13.75	227.1 -> 58.3	25	89	91	2.0	2.4	2.2	2.4	-0.2	0
Fenchlorphos	13.81	284.9 -> 270.0	10	89	93	0.8	1.6	2.4	3.0	-1.5	-1.4
Prometryn	13.85	241.1 -> 58.2	25	88	92	2.3	2.0	0.4	1.5	1.9	0.4
Aldrin	14.39	262.8 -> 193.1	20	79	84	1.4	3.0	2.2	2.2	-0.8	0.7
Bromacil	14.40	207.1 -> 54.1	60	86	91	0.8	1.9	2.1	2.3	-1.3	-0.3
Metolachlor	14.66	238.1 -> 162.1	20	86	94	0.3	1.0	1.6	1.5	-1.4	-0.4
Dicofol-p,p (degr.)	14.80	249.9 -> 139.1	35	85	89	1.2	1.8	3.0	1.1	-1.8	0.7
Parathion Ethyl	14.89	291.1 -> 109.1	20	91	107	3.0	2.6	5.0	3.6	-2	-1.1
DCPA (Dacthal)	14.90	300.8 -> 222.9	10	88	89	0.5	1.4	3.3	1.4	-2.7	0.1
MGK-264 I	15.38	164.1 -> 80.1	10	91	94	0.7	1.3	2.7	2.2	-2.0	-1.0
Cyprodinyl	15.73	223.9 -> 208.2	15	88	91	0.6	2.0	2.2	2.4	-1.6	-0.4
Heptachlor Epoxide	15.76	352.8 -> 262.9	20	83	88	1.8	4.1	4.7	2.0	-2.8	2.1
MGK-264 II	15.78	164.1 -> 98.1	10	89	93	0.8	3.0	1.2	3.2	-0.4	-0.2
Pendimethalin	15.96	252.1 -> 162.1	35	91	106	1.3	2.7	7.8	3.4	-6.5	-0.7
Folpet*	16.29	259.8 -> 130.1	10	36	34	22.6	11.4	12.3	5.8	10.3	5.6
Chlordane-Trans	16.54	372.8 -> 265.8	10	84	85	0.8	2.5	1.2	3.8	-0.4	-1.3
Endosulfan I	16.85	238.8 -> 204.0	40	87	90	1.2	2.9	3.6	2.4	-2.4	0.5
Chlordane-Cis	16.99	372.8 -> 265.8	10	86	87	3.5	3.5	3.6	3.2	-0.1	0.3
Napropamide	17.30	271.1 -> 72.1	50	86	90	0.9	1.2	1.1	0.8	-0.2	0.4
Dieldrin	17.50	262.7 -> 193.1	40	86	87	2.2	2.9	2.6	2.2	-0.4	0.7
DDE-p,p	17.70	318.0 -> 246.0	10	82	82	0.6	2.8	1.1	2.6	-0.5	0.2
Endrin	18.10	262.7 -> 193.1	20	82	88	1.7	3.8	1.4	2.7	0.3	1.1
Oxyfluorfen	18.14	252.1 -> 146.2	50	87	108	1.3	2.8	4.9	2.5	-3.7	0.3
Endosulfan II	18.35	238.8 -> 204.0	60	86	87	3.0	2.4	3.7	2.7	-0.6	-0.3
DDT-p,p	18.51	234.9 -> 165.1	20	92	90	10.0	7.3	2.1	4.2	7.9	3.1
DDD-p,p	18.71	234.9 -> 165.1	10	91	90	10.0	7.2	2.1	4.3	7.9	2.9
Oxadixyl	18.80	163.1 -> 132.2	35	87	90	0.4	1.2	0.6	1.1	-0.2	0
Carbophenothion	19.30	342.1 -> 157.1	20	91	100	2.1	2.8	3.0	1.9	-1.0	0.9
Endosulfan Sulfate	19.32	271.8 -> 237.0	20	89	95	1.3	2.4	1.8	2.0	-0.5	0.3
Piperonyl Butoxide	20.10	176.1 -> 103.1	15	92	99	1.1	2.6	1.1	1.4	0	1.2
Iprodione	20.40	313.9 -> 56.2	20	90	96	4.4	4.3	2.8	3.3	1.6	1.1
Methoxychlor-p,p	20.70	227.1 -> 169.2	60	84	94	10.0	6.6	1.1	2.2	8.9	4.4
Tetradifon	21.05	228.8 -> 79.0	40	87	90	1.8	3.3	3.0	1.4	-1.2	1.9
Fenarimol	21.90	219.1 -> 107.2	50	87	91	1.7	1.8	1.2	0.8	0.5	1.0
Cyfluthrin	23.18	226.1 -> 206.1	200	93	102	3.1	2.4	3.2	1.2	-0.1	1.2
Fenvalerate [RR,SS]	23.71	167.1 -> 125.1	22	87	101	5.2	4.6	3.8	3.0	1.4	1.6
Fenvalerate [RS,SR]	23.81	167.1 -> 125.1	28	86	101	5.0	4.3	3.6	3.4	1.4	0.9

* For complete recovery pH control must be used

Inter-day Precision

Stability Over 75 Injections with 1 vs. 2 QuEChERS d-SPE Steps



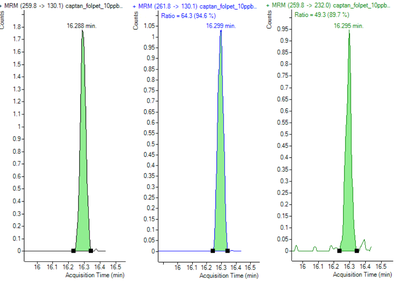
Dichlobenil, Propham, THPI, 1-Naphthol, Anthracene-D10, PCB, Diphenylamine, Chlorpropham, Ethalfluralin, Trilfluralin, alpha-BHC, Dicloran, HCB, Clomazone, Lindane, PCNB, Pronamide, Terbufos, Tefluthrin, Terbacil, Triallate, Ametryn, Chlorpyrifos Methyl, Prometryn, Vinclozolin, Aldrin, Bromacil, DCPA (Dacthal), Dicofol-p,p, Metolachlor, Cyprodinyl, Heptachlor Epoxide, MGK-264 I, Pendimethalin, Fonofos, Chlordane-Trans, Endosulfan I, DDE-p,p, Dieldrin, Napropamide, Endrin, Oxyfluorfen, Endosulfan II, DDD-p,p, DDT-p,p, Endosulfan Sulfate, Oxadixyl, Piperonyl Butoxide, Iprodione, Methoxychlor-p,p, Tetradifon, Fenarimol, Cyfluthrin, Fenvalerate [RS,SR], MGK-264 II, Fenvalerate [RR,SS], Chlordane-Cis, Fenchlorphos, Parathion Ethyl, Carbophenothion, Phorate

* Folpet not present in this standard

Consecutive injections of post-spiked 2xLOQ winter squash were made over approximately 36 hours. New GC columns and liners were used for each sequence of 75 injections. The MS source had over 740 injections by the end of the 2x d-SPE sequence. Responses are normalized to the first data point.

Highest %RSDs were obtained for carbophenothion (8.5), parathion ethyl (8.4) and phorate (7.0) in the 1x d-SPE sample. Overall averages of %RSDs for the 1x d-SPE and 2x d-SPE samples over 75 injections were 3.4 and 2.6, respectively.

Difficult to Analyze Pesticides: 10 ppb Folpet in Winter Squash



Folpet spiked at 10 ppb into 2x d-SPE non-acidified winter squash extract yielded a %RSD of 5.8 for 8 consecutive injections.

Conclusions

- The use of a second d-SPE step results in no loss in recovery
- RSDs did not increase significantly with the possible exception of pendimethalin, which may be stabilized by matrix components
- Performing two d-SPE steps in tandem may be a cost-effective and expeditious means of handing difficult samples

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