Optimizing Dispersive Solid Phase Extraction and Splitless Injection-Gas Chromatography for QuEChERS Fruit and Vegetable Extracts

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Introduction

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is a widely-adopted batch sample preparation method for analysis of pesticides in fruits, vegetables, and other food.

Dispersive solid phase extraction (dSPE), adding sorbent, e.g. primary secondary amine, directly to the QuEChERS extract, is used to provide a quick cleanup for samples prior to their analysis with gas and/or liquid chromatography.

The effects on recoveries from using different dSPE sorbents on strawberry extracts containing pesticides with a variety of chemical functionalities are shown. Preliminary results are illustrated from splitless injection optimization for a liner containing a CarboFritTM.

Standards and Samples

A custom mix of pesticides (listed in Table 1) was prepared at Restek. Pentachloronitrobenzene with formic acid was used as an internal standard.

Fresh organic strawberries were obtained from a local grocery store.

Sample Preparation

Strawberry extracts were produced using the latest QuEChERS method published by Anastassiades at www.quechers.com except for variations in dSPE.

The method in brief: 10g homogenized strawberries were vigorously shaken with 10mL acetonitrile for 1 min. The following salts were then added to the mixture: 4g $MgSO_4$, 1g NaCl, 1g trisodium citrate dihydrate, 0.5g disodium hydrogencitrate sesquihydrate, followed by shaking for 1 min. Phase separation by centrifugation produced an extract that was removed and used for dSPE experiments. Pesticides were spiked to give a final concentration of 200 ng/mL each before dSPE.

1 mL extracts were shaken for 30 sec (or 2 min for carbon) with 150mg $MgSO_4$ and the sorbent amounts below to effect dSPE. Centrifugation produced a clear extract for GC-MS analysis.

- 50mg PSA
- 50mg PSA, 50mg C18
- 50mg PSA, 50mg GCB
- PSA is primary secondary amine.
- GCB is graphitized carbon black.

GC-MS

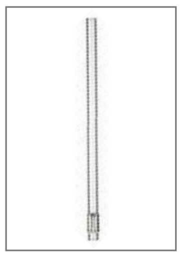
A 20m x 0.18mm x 0.14µm Rtx-CLPesticides2 GC column (Restek Corporation, USA) was installed in a Shimadzu QP-2010 Plus GC-MS system.

The mass spectrometer used electron ionization (70eV) with selected ion recording. The source temperature was 230°C.

One microliter splitless injections were performed by a Shimadzu AOC-20i autosampler using "mid" injection speed at the temperatures specified in the results. The liner used was deactivated glass, 5mm OD x 3.5mm ID x 95mm L. A single gooseneck at the bottom was approximately 12mm L (Figure 1). Packing materials were as specified in the results. The GC oven was held at 40°C for 1 min and then programmed at 12°C/min to 320°C.

Helium carrier flow was at a constant linear velocity of 40 cm/sec.

Figure 1. Single gooseneck splitless liner used for injection optimization. Packing materials (e.g. glass wool, CarboFritTM) were used to help vaporization.



Results and Discussion

Depending on the GC inlet design, liner configuration, and liner packing material (if used), experimental optimization is often necessary to produce acceptable results for splitless injection. This is especially critical for pesticide residue work because of the trace levels often found in food samples.

Injection speed, syringe needle type and length, valve time, and injection type (hot needle, solvent flush, etc) may also be important parameters to consider when optimizing a trace residue GC analysis.

CarboFritTM (Figure 2) is a packing material developed at Restek to overcome inertness issues associated with glass wool, while providing retention of higher molecular weight sample components that might lead to premature GC column failure.

Figure 2. CarboFritTM packing material for splitless injection.

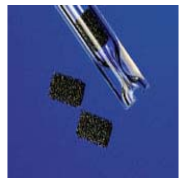


Table 1 shows that when the CarboFritTM is 35mm from the top of the liner, a few mm below where sample is expelled, higher injector temperatures may be necessary to increase response. This is related to pesticide volatility and becomes more dramatic for pesticides below the red line. Retention on carbonaceous material may be greater for compounds that can attain a planar shape, e.g. Thiabendazole (Figure 3). Caution must be used when raising injector temperature to increase response, because some pesticides are subject to degradation (values in red).

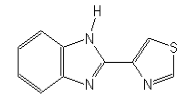
Table 1. Relative response factor results from different splitless temperatures and CarboFritTM positions for pesticides in PSA dSPE QuEChERS strawberry extracts. 35CF250, for example, is a liner with the CarboFritTM positioned 35mm from the top of the liner and a 250°C splitless injection.

Pesticide	RT (min)	m/z	35CF250	35CF275	35CF300	60CF250
Dichlorvos	9.50	185	3.42	3.36	3.35	3.09
Methamidophos	9.66	141	4.45	4.46	4.39	3.74
o-Phenylphenol	12.03	170	14.0	13.9	14.3	11.9
Acephate	12.66	136	4.38	3.44	1.22	4.06
Omethoate	13.69	156	3.22	3.63	3.66	2.73
Diazinon	14.75	304	1.46	1.65	1.91	1.71
Dimethoate	14.98	229	0.315	0.367	0.411	0.338
Chlorothalonil	15.70	266	2.75	3.29	3.46	2.89
Vinclozolin	15.87	285	1.79	1.85	2.05	1.73
Metalaxyl	16.21	206	3.60	3.84	4.19	3.24
Carbaryl	16.29	144	14.9	18.5	19.7	16.6
Malathion	16.60	173	3.54	4.44	4.43	4.96
Dichlofluanid	16.68	332	0.111	0.139	0.085	0.177
Thiabendazole	17.52	201	1.93	5.24	7.77	4.34
Captan	17.70	264	0.008	0.026	0.020	0.034
Folpet	17.77	260	0.020	0.018	0.011	0.170
Imazalil	18.18	215	0.503	1.45	1.95	1.37
Endrin	18.40	263	1.16	1.22	1.23	1.11
Myclobutanil	18.63	179	5.01	6.47	7.73	6.03
4,4'-DDT	19.08	235	1.26	2.52	1.87	6.00
Fenhexamid	19.23	301	0.315	0.556	0.689	0.623
Propargite 1	19.41	173	0.292	0.540	0.625	0.649
Bifenthrin	19.75	181	6.92	22.6	27.8	24.0
Dicofol	20.04	251	0.036	0.054	0.038	0.395
Iprodione	20.06	314	0.902	1.07	0.213	1.74
Fenpropathrin	20.22	181	1.61	4.51	5.43	4.65
cis-Permethrin	21.33	183	2.73	3.81	4.87	4.09
trans-Permethrin	21.47	183	3.98	9.54	12.3	10.6
Deltamethrin	23.75	253	0.008	0.665	1.91	1.39

Simply repositioning the CarboFritTM lower in the liner (60mm from the top) led to increased responses at lower temperatures, especially for those compounds that might be sensitive to degradation (values in blue).

PSA is the base sorbent used for dSPE cleanup of QuEChERS fruit and vegetable extracts because it removes organic acids and sugars that might act as interferences.

Figure 3. Structure of the fungicide Thiabendazole.



A pesticide-spiked strawberry extract (200 ng/mL) subjected to dSPE with PSA was used to generate one-point calibration curves. Spiked strawberry extracts subjected to additional dSPE sorbents were analyzed and the results versus PSA dSPE are shown as percent recoveries in Table 2. C18 is suggested for use when samples might contain fats, not an issue for a strawberry extract, but it was important to verify that gross losses of more hydrophobic pesticides (e.g. Endrin and DDT) would not occur. GCB is used to remove pigments, and when treated, the red strawberry extract became clear. However, GCB can also have a negative effect on certain pesticides, especially those that can assume a planar shape like Chlorothalonil and Thiabendazole (values in red).

Table 2. Pesticide recoveries in percent for strawberry extracts treated with C18 and GCB dSPE versus PSA only. The injector liner packing was quartz wool. **NOTE**: The single gooseneck liner used in this work has the gooseneck too low for the heated zone in the Shimadzu 2010 GC. The liner is being redesigned.

Pesticide	CAS Number	Action	Classification	C18	GCB
Dichlorvos	62-73-7	Insecticide	Organophosphorus	111	116
Methamidophos	10265-92-6	Insecticide	Organophosphorus	105	107
o-Phenylphenol	90-43-7	Fungicide	Unclassified	106	97
Acephate	30560-19-1	Insecticide	Organophosphorus	128	147
Omethoate	1113-02-6	Insecticide	Organophosphorus	120	119
Diazinon	333-41-5	Insecticide	Organophosphorus	108	127
Dimethoate	60-51-5	Insecticide	Organophosphorus	124	151
Chlorothalonil	1897-45-6	Fungicide	Organochlorine	125	13
Vinclozolin	50471-44-8	Fungicide	Organochlorine	102	98
Metalaxyl	57837-19-1	Fungicide	Organonitrogen	105	117
Carbaryl	63-25-2	Insecticide	Carbamate	114	111
Malathion	121-75-5	Insecticide	Organophosphorus	124	160
Dichlofluanid	1085-98-9	Fungicide	Organohalogen	122	103
Thiabendazole	148-79-8	Fungicide	Organonitrogen	88	14
Captan	133-06-2	Fungicide	Organochlorine	88	91
Folpet	133-07-3	Fungicide	Organochlorine	108	63
Imazalil	35554-44-0	Fungicide	Organonitrogen	115	95
Endrin	72-20-8	Insecticide	Organochlorine	104	101
Myclobutanil	88671-89-0	Fungicide	Organonitrogen	119	114
4,4'-DDT	50-29-3	Insecticide	Organochlorine	102	95
Fenhexamid	126833-17-8	Fungicide	Organochlorine	118	77
Propargite 1	2312-35-8	Acaricide	Organosulfur	110	95
Bifenthrin	82657-04-3	Insecticide	Pyrethroid	106	81
Dicofol	115-32-2	Acaricide	Organochlorine	98	54
Iprodione	36734-19-7	Fungicide	Organonitrogen	118	90
Fenpropathrin	39515-41-8	Insecticide	Pyrethroid	113	96
cis-Permethrin	52645-53-1	Insecticide	Pyrethroid	106	65
trans-Permethrin	51877-74-8	Insecticide	Pyrethroid	109	71
Deltamethrin	52918-63-5	Insecticide	Pyrethroid	97	52

Conclusions

- Experimental optimization of splitless injection with careful attention to liner design, injector temperature, and packing material type/placement is necessary for improving response in pesticide analysis.
- Carbon in dSPE should be used with caution when analyzing certain pesticides.

References

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, J. AOAC Int., 86 (2003) 412.

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