



Application Note: Multi-Residue Pesticides in Fruit and Vegetables (9/16/08)

Product: DPX-Q (5 mL)

INTRODUCTION

The analysis of pesticides in fruit and vegetables is very important in the field of food safety. To ensure levels of toxic pesticides are below tolerance levels and are safe to ingest, routine and comprehensive testing must be performed. The biggest drawback to this analysis has been sample preparation.

Recently, there has been a great amount of interest in QuEChERS¹⁻², which stands for quick, easy, cheap, effective, rugged and safe. This method is used to remove fatty acid components rather than to extract and isolate the pesticides. The advantage of this method is that it is comprehensive, providing very high recoveries for almost all pesticides. The disadvantage of this method is that the resultant sample solutions are relatively "dirty", and as a result there have been numerous modifications and variations of the original method. Some of the different methods include the use of dispersive tubes or cartridges, and some use graphite or other additives (like C₁₈) to remove sample matrix interferences.

In this study, the QuEChERS concept is used in a relatively new solid-phase extraction method called disposable pipette extraction (DPX). The DPX tip has loosely contained solid-phase sorbent contained inside a pipette tip through the use of a screen and upper barrier. The main advantages of DPX are its speed and ease of use, taking only seconds to mix sample solutions with the solid-phase sorbent. The advantage of the DPX-Q over other QuEChERS products is that the screen of the DPX tip permits dispersive extractions without the need for centrifugation for the "clean up". The screen of the DPX tip acts as a filter removing the sorbent and salt particulate matter from the solution, thereby providing a convenient means of conducting the QuEChERS extraction.

EXPERIMENTAL

Initial Sample Preparation

- Add 15 g blended sample (carrots) and 15 mL acetonitrile into 50 mL centrifuge tube
- Add 1.5 g NaCl and 6.0 g MgSO₄
- Cap and shake tube vigorously for a few minutes; let stand app. 10 minutes
- Centrifuge at 3,000 rpm for 10 minutes
- Transfer 2.5 mL of the acetonitrile solution (supernatant) for the manual DPX-Q method.

Manual DPX-Q extraction steps

1. If using an internal standard, spike the standard into the 2.5 mL of acetonitrile solution
2. Aspirate the acetonitrile solution in and out of the DPX-Q tip using an attached syringe.
3. Repeat this mixing 2-3 times.
4. Dispense the solution following the final DPX-QuEChERS extraction into a GC vial, cap and inject into the GC.
5. **Alternatively**, dispense the DPX-Q solution into a small vial and concentrate to 0.5 mL of acetonitrile (or another solvent such as toluene) and then transfer to a GC vial and inject.



DPX-Q tips: 5 mL DPX tips containing MgSO₄, PSA and graphitized carbon black (GCB)

Instrumentation.

The GC/MS was an Agilent Technologies 6890 GC with 5972A MSD (inert XL). This instrument utilized the following conditions:

GC column: 30 m DB-1701 (J&W Scientific), 0.25 mm ID, df = 0.25um
carrier gas: He at constant flow of 1 mL/min
oven: initial temp at 80 °C for 1 min, ramp at 20 C°/min to 300 °C, hold 7 min (19 min run)
inlet temperature: 250 °C
injection: 2 µL using a HP6890 Series Injector

Table 1. Parameters for the MS analysis by SIM for OC pesticides.

Group #	Time (min)	pesticides	Monitored ions (m/z)	Dwell (msec)
1	8.50	Alpha-BHC	181, 219, 111	30
2	9.20	Gamma-BHC, Beta-BHC, Heptachlor	100, 109, 181, 219, 237, 272	30
3	9.90	Delta-BHC, aldrin	66, 181, 109, 219, 293,263	30
4	10.50	Heptachlor epoxide	81, 263, 353	30
5	11.00	Endosulfan I, 4,4'-DDE, Dieldrin	79, 81, 176, 195, 241, 246, 263, 318,339	30
6	11.80	Endrin, 4,4'-DDD	81, 165, 235, 237, 263,345	30
7	12.20	Endosulfan II, 4,4'-DDT	165,170, 195, 199, 235, 237,	30
8	12.60	Endrin aldehyde, Endosulfan sulfate	67, 237, 250, 272, 387, 345	30
9	13.50	Methoxychlor	227, 228, 346	30

Table 2. Parameters for the MS analysis by SIM for OP pesticides.

Group #	Time (min)	pesticides	Monitored ions (m/z)	Dwell (msec)
1	5.40	Dichlorphos	79, 109, 185	30
2	6.80	Mevinphos	109, 127, 92	30
3	8.00	Ethoprophos	97, 139, 158	30
4	8.55	Phorate, Demeton-S, Diazinon	60, 75, 88, 121, 137, 152, 170, 179, 260	30
5	9.25	Disulfoton	88, 97, 274	30
6	9.80	Ronnel, Methyl parathion, Trichloronat, Chlorpyrifos	97, 109, 125, 197, 263, 269, 285, 287, 297, 314	20
7	10.50	Fenthion	109, 125, 278	30
8	10.90	Merphos, Tokuthion, Stirofos	57, 109, 113, 162, 169, 267, 314, 329, 331	30
9	12.00	Bolstar, Fensulfothion	139, 141, 156, 293, 322, 308	30
10	16.80	Coumaphos	109, 226, 362,	30

RESULTS AND DISCUSSION

The initial sample preparation used the same method that is delineated in QuEChERS¹⁻². The sample was "shaken" in a ratio of 1:1 with acetonitrile in order to achieve good sensitivity. The addition of salt is used to separate the acetonitrile layer from the water. The concept of



QuEChERS is to then remove the sample matrix. This is readily accomplished using the DPX-Q tips and an attached disposable syringe (or pipette). The acetonitrile solution is aspirated in and out of the DPX-Q tip a few times, then the solution is transferred into a GC vial and injected.

For higher recovery, the solution can be concentrated to a lower volume, or solvent exchanged using a "keeper" solvent such as toluene to stabilize the pesticides ("keeper" solvent such as toluene). It is also possible to concentrate the analytes directly into the GC inlet by using a PTV inlet (such as the GERSTEL CIS), which would reduce time and eliminate solvent evaporation.

The DPX-Q method takes only 90 seconds to perform; there are no wash steps or elution steps. The acetonitrile solution is simply mixed with the sorbent a few times to remove fatty acid components, sample matrix components and water.

Results from the analysis of organochlorine and organophosphate pesticides are shown in Tables 3 and 4, respectively. The recoveries were very good for all of the pesticides analyzed with only one exception. One of the OC pesticides, endrin aldehyde, had interference with a sample matrix component in oranges, and so it could not be analyzed using the procedure delineated in the experimental section. Changing the type of GC column or ions analyzed may eliminate the interference.

It should be noted that the extracts appear "clean" with the use of the graphitized carbon. However, the GC inlet liners still obtain a build up of residue and must be changed routinely.

Table 3. Statistical results for organochlorine pesticides extracted from carrots and oranges.

OC Pesticides	%Recovery in carrot	%RSD in carrot	%Recovery in orange	%RSD in orange
α -BHC	87.78	3.93	92.84	2.71
γ -BHC	88.73	3.57	89.42	0.69
β -BHC	88.42	3.67	92.01	2.08
δ -BHC	104.85	12.65	92.39	2.50
Heptachlor	85.68	7.22	89.47	4.32
Aldrin	90.84	5.26	92.10	3.47
Heptachlor epoxide	84.57	5.13	94.26	8.57
Endosulfan I	90.79	4.87	105.23	4.18
p,p'-DDE	88.23	3.38	92.59	2.41
Diendrin	91.77	3.79	99.07	3.15
Endrin	94.99	6.11	93.46	0.93
p,p'-DDD	88.67	5.49	95.60	3.82
Endosulfan II	91.88	3.82	94.53	3.90
p,p'-DDT	89.38	4.48	84.78	3.52
Endrin aldehyde	84.53	3.08		
Endosulfan sulfate	97.76	6.29	97.97	1.77
Methoxychlor	89.30	5.02	83.01	3.83



Table 4. Statistical results for organophosphate pesticides extracted from carrots and oranges.

OP Pesticides	%Recovery in carrot	%RSD in carrot	%Recovery in orange	%RSD in orange
Dichlorphos	86.98	5.01	93.05	3.05
Mevinphos	91.15	4.15	113.29	2.62
Ethoprophos	88.12	3.93	97.43	1.12
Phorate	89.01	3.28	89.04	2.64
Demeton-S	88.09	7.16	108.60	5.17
Diazinone	81.77	7.48	90.36	2.18
Disulfoton	89.42	4.19	85.71	3.92
Ronnel	90.11	7.06	88.92	3.58
Parathion-methyl	85.50	4.99	104.43	3.48
Trichloronat	85.60	3.08	83.63	1.22
Chlorpyrifos	80.69	2.43	72.64	2.94
Fenthion	86.16	4.00	91.98	2.29
Merphos	88.34	5.41	99.24	4.83
Tokuthion	88.35	3.68	88.57	1.86
Stirofos	94.75	11.08	103.66	10.24
Bolstar	89.09	3.40	85.61	2.12
Fensulfothion	89.64	4.65	106.64	2.88
Coumaphos	76.91	4.52	108.66	2.73

It has been found that the QuEChERS products provide high recoveries of polar pesticides such as acephate and methamidophos. These compounds are difficult to isolate and extract due to their high polarity, but these analytes can be readily detected using QuEChERS because this method does not attempt to bind the compounds of interest. Using the DPX-Q tips, good recoveries of these compounds were obtained in carrots and oranges (Table 5).

Table 5. Statistical results for miscellaneous pesticides, including chlorothalonil and acephate.

Misc. Pesticides	%Recovery in carrot	%RSD in carrot	%Recovery in orange	%RSD in orange
Methamidophos	66.92	3.53	74.97	1.78
Acephate	71.80	9.97	84.92	2.12
Captan	77.75	8.60	85.39	3.95
Chlorothalonil	79.57	2.77	62.47	8.21
Thiabendazole	88.45	1.48	70.12	2.44

Other compounds that have been difficult to analyze by QuEChERS include chlorothalonil, captan and thiabendazole. These compounds were also extracted with good recoveries using the



DPX-Q tips (Table 5). It is noted that the DPX-RP tips³ (discussed in a previous application note) appear to provide better recoveries of chlorothalonil and captan, and this product may be used as a complimentary method to DPX-Q.

CONCLUSION

This method proves to be very reproducible and efficient for analysis of pesticides in fruit and vegetables. This study demonstrates a rapid, convenient and efficient method for comprehensive pesticide screening. DPX-Q provides high recoveries of all pesticides tested, including polar pesticides such as acephate.

Further research focuses on "automated" DPX-Q, where the "cleanup" method is performed by a GERSTEL MPS-2 and the resultant eluent is injected into a GERSTEL CIS for large volume injection for improved sensitivity.

REFERENCES

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