



Application Note: Analysis of Pesticides in Fruit and Vegetables (7/06/08)

Product: DPX-RP (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 QuEChERS, 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). Another disadvantage is that the modified methods require the evaporation of over 15 mL of organic solvent into the atmosphere for every sample processed.

In this study, a relatively new solid-phase extraction method called disposable pipette extraction (DPX) was used to extract pesticides from fruit and vegetables. The main advantage of DPX is its speed, taking only a few short minutes to extract analytes from sample matrices. By using a mixing step, the extraction efficiencies are optimized and therefore less sorbent material is required for high recoveries. This results in the need for less elution solvent volumes, which subsequently can be used to provide a "built-in" concentration step. In this study, extracts and chromatographic analyses were performed without any solvent evaporation by using the DPX-RP product for reverse phase mechanisms.

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 1 mL of the acetonitrile solution (supernatant) for the manual DPX method.

Manual DPX extraction steps

1. Add 2.4 mL deionized water and 0.8 mL saturated NaCl to 1 mL of the sample solution and vortex mix
2. Using the syringe device, aspirate the entire sample solution into the 5 mL DPX-RP tip and app. 5 mL of air to mix the solution with air bubbles
3. Wait approximately 30 seconds
4. Dispense back into the test tube
5. Aspirate 0.5 mL of DI water (from a test tube) and app. 3 mL of air to mix the solution
6. Wait 10 seconds and dispense to waste



7. Remove the syringe device and add 0.7 mL of 50/50 hexanes-ethyl acetate to the top of DPX tip
8. Re-attach the syringe device and dispense the eluent into a small labeled test tube
9. Remove the bottom layer (app. 100 uL) of water using a disposable Pasteur pipette (or pass the solution through a small amount of Na₂SO₄ using a Pasteur pipette with a plug of glass wool to remove the water)
10. Add 50 uL of external standard (methyl chlorpyrifos) into the eluent
11. Transfer the final eluent (app. final volume of 0.5 mL) into a GC vial insert and place into a GC vial
12. Cap, place on the autosampler and inject into the GC

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 QUECHERS. 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. With DPX, the goal is to extract and isolate the analytes of interest onto the sorbent of the DPX tips. This is accomplished by adding water and salt to decrease the concentration of organic solvent using reversed phase mechanisms (similar to common HPLC methods).

By incorporating "mixing" of the sorbent, very high extraction efficiencies can be achieved with low mass of sorbent. This low amount of sorbent permits the extraction to require much less solvent, thereby eliminating the need for solvent evaporation. Without solvent evaporation, the extractions are much faster and are more environmentally friendly.

Results from the analysis of organochlorine and organophosphate pesticides are shown in Tables 3 and 4, respectively. The results are exceptional for these pesticides, approaching around 100% recoveries for most of them. By using hexanes-ethyl acetate for elution, the water is removed from the eluent due to being immiscible (like common liquid-liquid extractions). This solvent is an excellent "keeper" solvent (little to no sample degradation) that is ideally suited for GC analysis with various types of detectors. This means that both the solvent exchange and the concentration steps are accomplished at one time. It should be noted that the reason the final eluent volumes are less than the amount used in the extractions is due to the fact that the sorbent absorbs some of the solvent.

Similar results were obtained for oranges and spinach. The recoveries do not appear to be greatly affected by the matrix with this DPX method.

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

OC Pesticide	%Recov.	%RSD
α -BHC	105.6	3.4
γ -BHC	101.7	2.2
β -BHC	101.3	3.2
δ -BHC	112.3	4.3
Heptachlor	97.8	4.6
Aldrin	95.5	3.8
Heptachlor epoxide	106.4	1.7
Endosulfan I	102.5	2.8
p,p'-DDE	91.6	5.0
Dieldrin	102.9	2.7
Endrin	97.2	3.7
p,p'-DDD	98.8	1.8
Endosulfan II	99.0	2.0
p,p'-DDT	89.1	8.4
Endrin aldehyde	96.2	1.4
Endosulfan sulfate	101.2	5.7
Methoxychlor	94.5	4.8

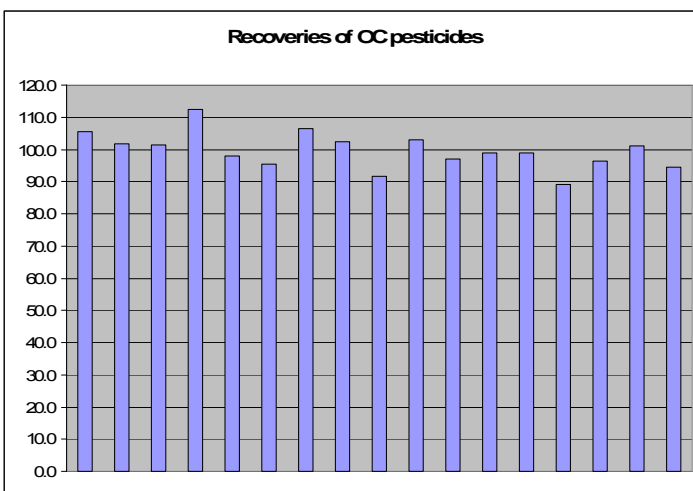




Table 4. Statistical results for organophosphate pesticides extracted from carrots.

OP pesticide	% Recov.	%RSD
Dichlorphos	57.9	3.8
Mevinphos	25.7	6.5
Ethoprophos	88.8	2.6
Phorate	95.6	1.5
Demeton-S	72.4	3.0
Diazinon	98.1	3.5
Disulfoton	89.3	2.0
Ronnel	96.8	2.0
Parathion-methyl	89.4	3.4
Trichloronat	87.2	6.3
Chlorpyrifos	89.6	7.6
Fenthion	88.4	6.2
Merphos	99.7	3.7
Tokuthion	88.7	3.4
Stirofos	86.8	5.0
Bolstar	92.0	2.5
Fensulfothion	81.3	5.0
Coumaphos	94.3	5.0

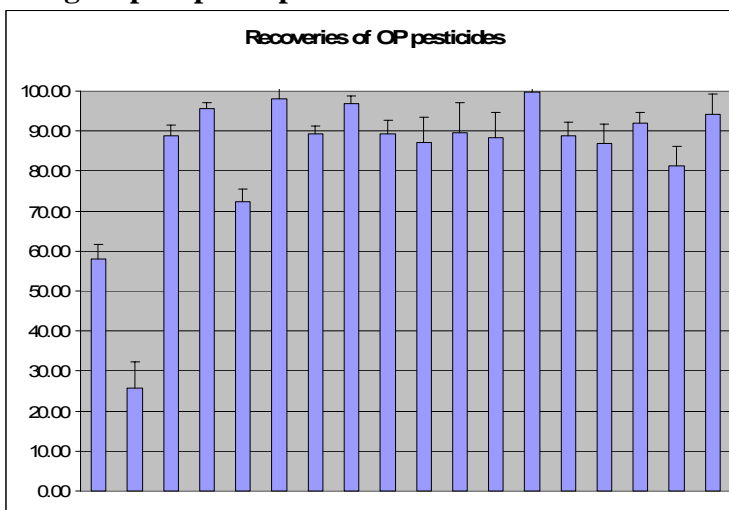
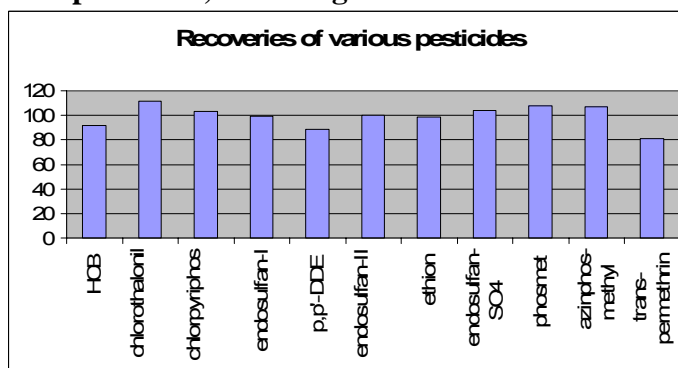


Table 5. Statistical results for various pesticides, including chlorothalonil.

Pesticide	%Recov.	%RSD
HCB	91.7	4.5
chlorothalonil	111.9	4.9
chlorpyrifos	103.2	3.5
endosulfan-I	99.2	4.4
p,p'-DDE	89	1
endosulfan-II	99.9	2.9
ethion	98.7	3.6
endosulfan-SO4	103.8	1.7
phosmet	108	0.2
azinphos-methyl	107.1	1.5
trans-permethrin	81	8.3



Another noteworthy observation is that this DPX method provides efficient and reproducible recoveries of chlorothalonil and trans-permethrin, a pyrethroid insecticide (Table 5).

Chlorothalonil is apparently difficult to analyze using the QuEChERS method, so the DPX-RP method should be a viable alternative for this analyte in particular. The LOD for chlorothalonil was found to be less than 10 ppb, and the chromatograms shown in Fig. 1 were recorded after approximately 50 extracts of carrot samples.

It is obvious that DPX-RP works very well for most of the pesticides. However, low recoveries were obtained for the most polar organophosphate pesticides. It is actually possible to predict the recovery of the pesticides based on its polarity. In Fig. 2, a plot of the recovery of the OP pesticide (in carrots or oranges) vs. its log P value is shown. The polarity of the pesticide is indicated by its K_{ow} value, which refers to the partitioning in water and 1-octanol. Note that the low recovery is only found for the very polar pesticides.

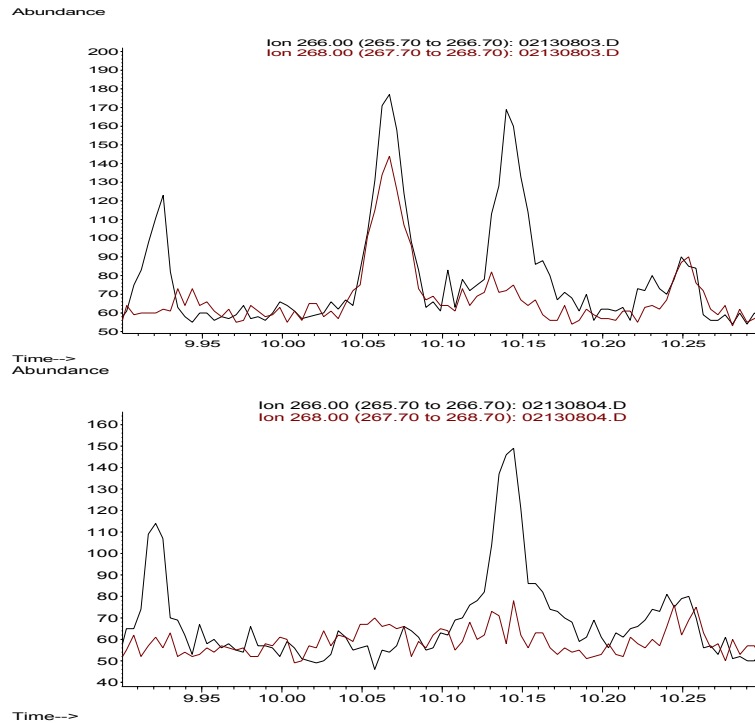


Figure 1. Extracted ion chromatograms (ions $m/z = 266$ and 268) of 10 ppb chlorothalonil spiked in carrots (TOP) and blank carrot matrix. The chlorothalonil peak at 10.06 min has a signal-to-noise ratio of app. 12.

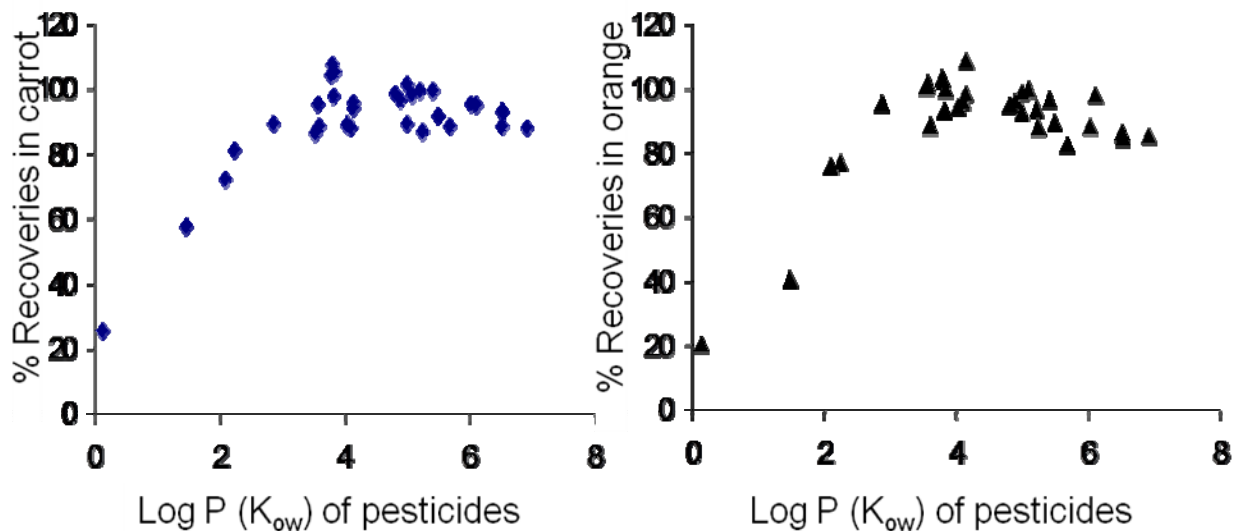


Fig. 2. Graphs of % recovery of organophosphate pesticides vs. the log P value (referring to the degree of polarity) of the pesticides. The polarity is based on the equilibrium partition constant of water and 1-octanol. The recovery is low only for very polar pesticides, with log P values less than approximately 1.8.



CONCLUSION

This method proves to be very reproducible and efficient for analyzing numerous pesticides in fruit and vegetables. This study demonstrates a rapid, convenient and efficient method for comprehensive pesticide screening. The sensitivity for the analysis is very good, though the LODs may be lowered by extracting larger sample volumes through multiple DPX extractions. This research demonstrates a rapid method of analysis of pesticides with high recoveries. This method required no solvent evaporation and used very low volumes of organic solvent, resulting in a “greener” chemistry.

Further research of a DPX tip for QuEChERS, called the DPX-Q, has been conducted and can be found in a subsequent application note. This DPX-Q provides high recoveries of almost all pesticides tested, including polar pesticides such as acephate.

REFERENCES

W. E. Brewer, H. Guan, S. R. Garris, C. Craft and S. L. Morgan, “Disposable pipette extraction of pesticides in fruit and vegetables”, *manuscript in preparation*.

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