Analysis of Pesticides in Fruits and Vegetables

HIGHLIGHTS: Highly reproducible results with reduced sample prep. time RP - XTR

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 QuEChERS (quick, easy, cheap, effective, rugged and safe) 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 graphitized carbon black (GCB) or other additives (like C18).

In this study, Dispersive Pipette XTRaction technology was used for solid phase extraction of pesticides from fruit and vegetables. These tips contain loose or dispersive sorbent material. Simple aspirate and dispense steps are used to mix the sample solution with sorbent inside the tip. The mixing results in increased extraction efficiencies and less sorbent material is required. Extracts and chromatographic analyses were performed using 5 mL XTR tips with Reverse Phase (RP) sorbent.

MATERIALS AND METHODS

The initial sample preparation used the same method that is delineated in QuEChERS outlined in table 1. 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 dispersive pipette extraction method isolates the analytes of interest with RP sorbent. Water and salt is added to decrease the concentration of organic solvent.

Aspirate and dispense steps mix the sorbent, and sample solution resulting in increased extraction efficiencies 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 and reducing sample prep. time. Sample extraction was performed following the method in Table 2 on a 5 mL Pneumatic Extractor.

Figure 1. DPX 5 mL Pneumatic Extractor is a syringe device that operates under gas to create positive and negative pressure to aspirate and dispense solutions in and out of 5 mL tips

Analysis was performed on a GC/MS 6890 GC with 5972A MSD (inert XL) from Agilent Technologies. This instrument utilized the following conditions:

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

Table 1. Sample Preparation

1	Add 15 g of blended carrot samples, 15 mL of acetonitrile, 1.5 g NaCL and 6.0 g MgSO4 to a centrifuge tube
2	Shake sample solution vigouroulsy for a few minutes; let stand for ~ 10 minutes and then centrifuge at 3,000 rpm for ~ 10 minutes
3	Transfer 1 mL of supernatant solution to test tubes on a 5 mL Pneumatic Extractor

Table 2. Sample Extraction Method - Final eluent volumes are less than the amount used in the extractions because the sorbent absorbs some of the solvent

Pretreatment	• Add 2.4 mL deionized water and 0.8 mL saturated NaCl to 1 mL of the sample solution and vortex mix
Extraction	• Using RP-XTR tips aspirate sample solution and ~ 5 mL of air to mix. Wait ~ 30 seconds, dispense • Aspirate 0.5 mL of DI water and ~ 3 mL of air to mix. Wait 10 seconds then dispense to waste
Elution	• Remove the syringe device and add 0.7 mL of 50/50 hexanes-ethyl acetate to the top of the tip • Re-attach the syringe device and dispense the eluent into another test tube.
Transfer	• Remove the bottom layer (~ 100 µL) of water • Add 50 mL of external standard (methyl chlorpyrifos)
Prep for injection	• Transfer final eluent (~ 0.5 mL) to GC vial, cap and inject

Table 3. MS Parameters by SIM for OC Pesticides

	Time	Pesticides	Monitored ions (m/z)	Dwell (msec)
1	8.5	Aplha-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	11.0	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 4. MS Parameters by SIM for OP Pesticides

	Time	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	30
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

Results from the analysis of organochlorine and organophosphate pesticides are shown in Figure 2 and 3, 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 (like common liquid-liquid extractions). Little to no sample degradation is shown when using this solvent, making it ideally suited for GC analysis with various types of detectors. Both the solvent exchange and the concentration steps can be accomplished at one time. Similar results were obtained for oranges and spinach. The recoveries do not appear to be greatly affected by the matrix with this method.

Chlorothalonil is difficult to analyze using the QuEChERS method but the dispersive pipette extraction method provides efficient and reproducible recoveries of chlorothalonil and trans-permethrin, a pyrethroid insecticide (Figure 4). The LOD for chlorothalonil was found to be less than 10 ppb, and the chromatograms shown in Figure. 5 were recorded after ~ 50 extracts of carrot samples.

Low recoveries were obtained for the most polar organophosphate pesticides. Prediction for the recovery of pesticides can be made based on its polarity. Figure. 6 shows 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 Kow value, which refers to the partitioning in water and 1-octanol. Note that the low recovery is only found for the very polar pesticides.

CONCLUSIONS

This method proves to be very reproducible and efficient for analyzing numerous pesticides in fruit and vegetables. This study demonstrates a rapid, method for comprehensive pesticide screening. The sensitivity for the analysis is very good, and the LODs may be lowered by using larger sample volumes with multiple extractions.

This method required no solvent evaporation and used very low volumes of organic solvent, resulting in less waste for an environmentally friendly method.

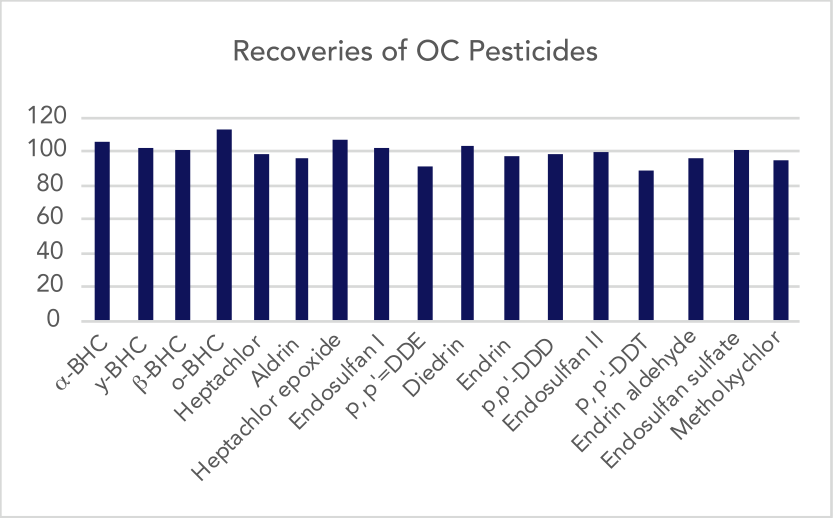

Figure 2. Statistical results for organochlorine pesticides extracted from carrots.

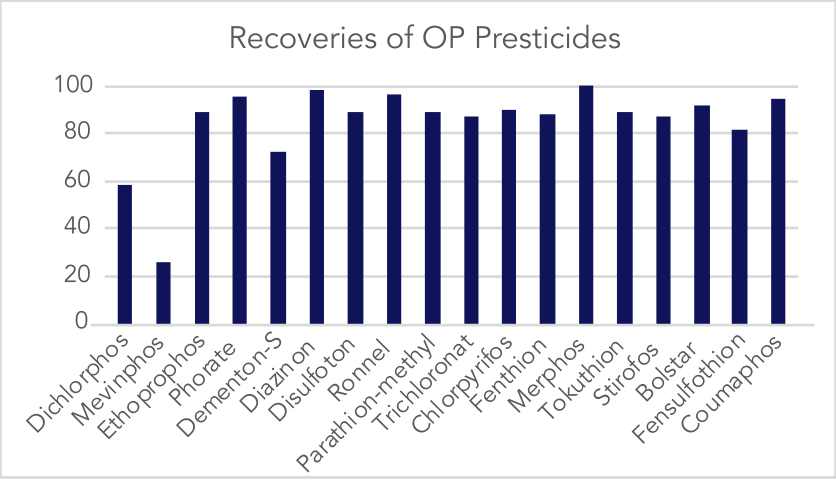

Figure 3. Statistical results for organophosphate pesticides extracted from carrots.

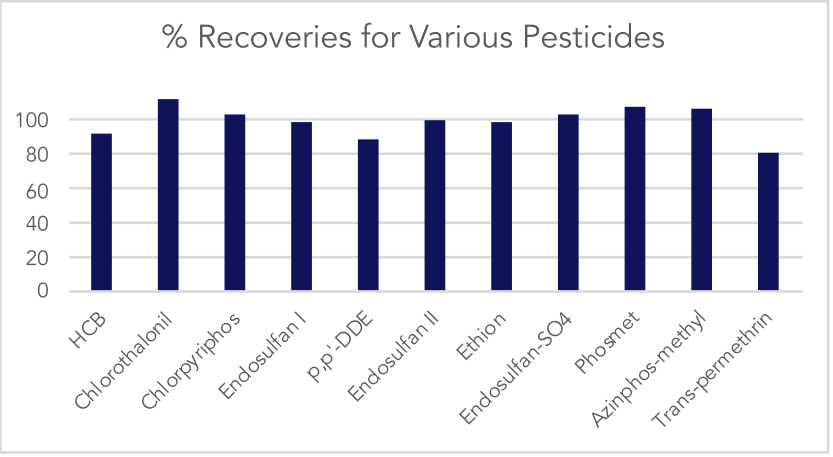

Figure 4. Statistical results for various pesticides, including chlorothalonil.

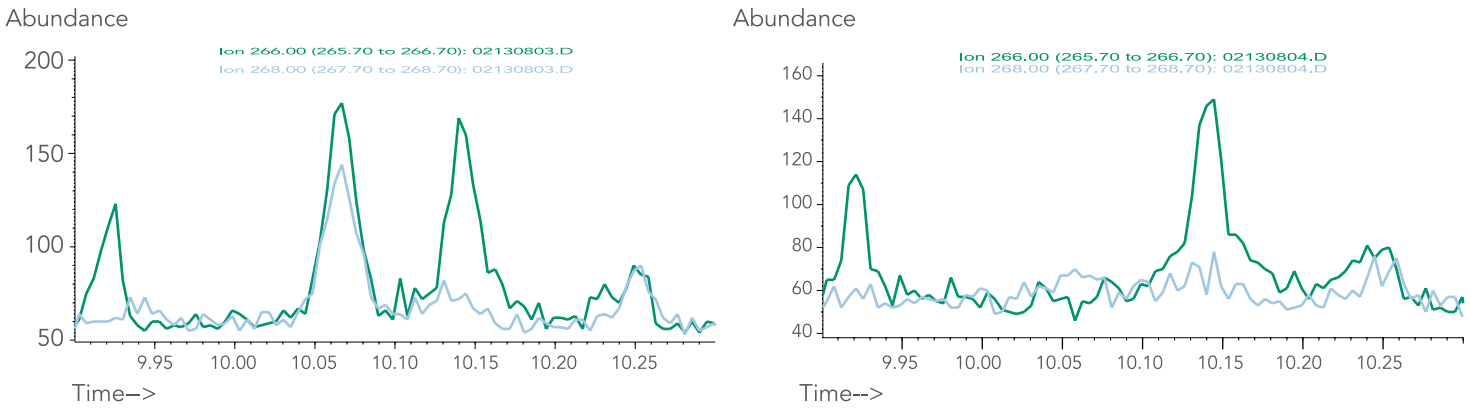

Figure 5. Extracted ion chromatograms (ions m/z = 266 and 268) of 10 ppb chlorothalonil spiked in carrots (TOP) and blank carrot matrix. The chlorothalonil peak at 10.06 min has a signal-to-noise ratio of ~ 12.

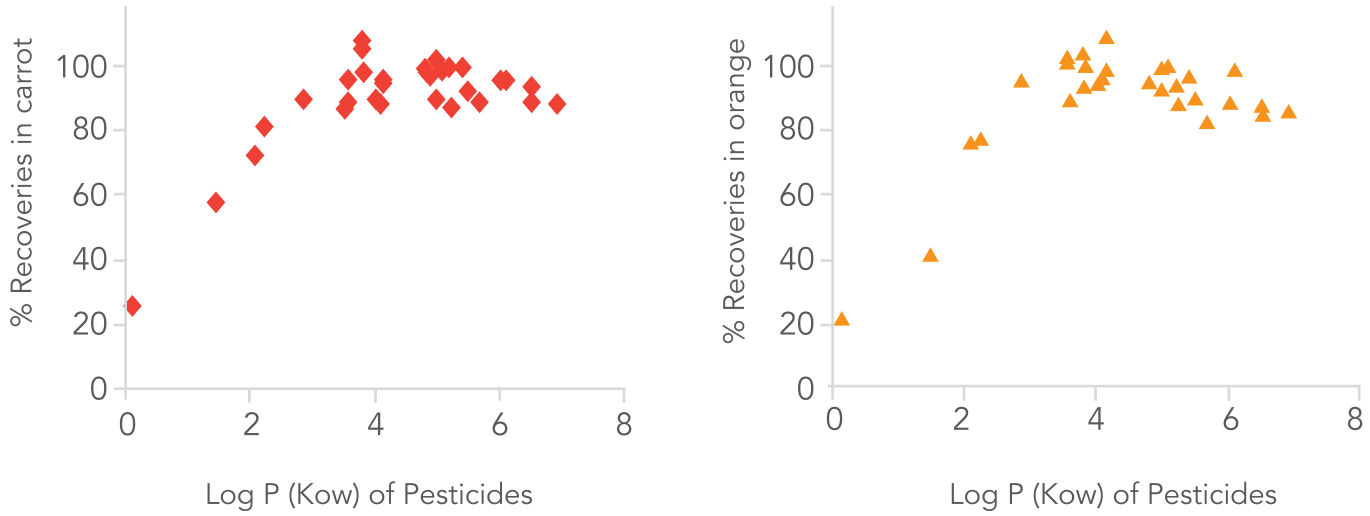

Figure 6. Graphs of % recovery of organophosphate pesticides vs. the log P value (referring to the degree of polarity) of the pesticides. The polarity is based on the equilibrium partition constant of water and 1-octanol. The recovery is low only for very polar pesticides, with log P values less than approximately 1.8.

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