How DPX and Micro Luke Works at SCDA to Screen Produce for Pesticide Residue

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Abstract

A Modified Micro Luke Method and Disposable Pipette Extraction (DPX) method is described that is used for the extraction of polar and nonpolar pesticides, respectively. This method takes advantage of the use of selective detectors for gas chromatography (GC) such as a nitrogen-phosphorous detector (NPD) and electron capture detector (ECD). Also, this method can be used with mass spectrometry (MS) for GC (or high performance liquid chromatography (HPLC) following solvent exchange).

The extractions are first processed using acetone, and one portion of the acetone solution undergoes a rapid liquid-liquid extraction for the polar pesticides, such as organophospates and organo-nitrogen pesticides. The 2nd portion of acetone is processed using reversed phase mechanisms with DPX tips. The time-consuming column chromatography in the Luke Method for GC-ECD analysis of organochlorine pesticides is not required. The DPX extracts result in chromatograms that have low background interferences for a wide variety of fruit and vegetables. In fact, the ECD chromatograms obtained by using DPX are comparable to those obtained by the Luke Method.

The results using the combined Modified Micro Luke and DPX method with the standard Modified Luke Method (using column chromatography cleanup for ECD) are shown. Improved recoveries for the nonpolar pesticides are shown using the DPX method. In addition, sample throughput has been nearly doubled while reducing costs for the analysis.

Key Words

Gas Chromatography; Sample Preparation; DPX; Pesticides

Introduction

Over the Last four years the South Carolina Department of Agriculture (SCDA) has been working on a new rapid extraction method called DPX to screen produce and other matrices for Pesticide Residue. We compared the DPX to the Luke and the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) Methods by running samples of incurred Positive and Spiked produce by all three methods.

QuEChERS gave good recoveries for almost all pesticides, but the extracts were too dirty for ECD chromatograms. Interferences were found to be too high to identify numerous pesticides in many sample matrices.

DPX worked great for nonpolar and slightly polar pesticides; however, it gave poor recoveries for very polar pesticides (such as acephate or thiabendazole).

We developed the Micro Luke Method to augment DPX so we could extract the polar pesticides as well as the non-polar. In this case, the time-consuming column chromatography cleanup for ECD analysis was replaced with DPX. The traditional liquid-liquid extraction for polar pesticides and NPD analysis was still utilized to analyze the problematic pesticides such as acephate.

The results of these studies have been excellent and SCDA is currently using DPX and a Modified Micro Luke Method in our Chemical Residue Lab at SCDA. With funds being cut on all levels, SCDA has been able to do twice as many samples for half the cost and detect more compounds at lower levels. Using Acetone instead of Acetonitrile is another advantage, especially during the shortage and with the higher cost for acetonitrile.

Experimental

DPX procedure ---- ECD/MSD method

1. Place 2.5 mls of acetone extract into a glass test tube
2. Add 6.0 mls of D.I. water and 2.5 mls of saturated NaCl
3. Draw in about 3.5 mls of total solution to a DPX-RP-5 tip, draw in about 10 mls of air to mix.
4. Stand for 30 sec. to 1 min, then dispense into a waste container
5. Repeat two more times until all the solution is drawn the same tip
6. Add 1.0 ml of D.I. water to DPX top and dispense to waste
7. Add 0.7 ml of Hexane-ethyl acetate (1+1, v/v) to DPX top and elute to GC vial transfer upper layer to vial insert and inject 1ul.

Micro Luke procedure – NPD / MSD method

1. Place 2.5 ml of acetone extract into a 50ml Teflon C-tube w/cap
2. Add 2.5 ml of saturated NaCl
3. Add 2.5 ml of petroleum ether
4. Add 7.5 mls of methylene chloride, shake/vent/vortex 20 sec.
5. Add ~ 14.5 grams sodium sulfate, shake/vent/vortex 10 sec.
6. Centrifuge for 5-10 minutes, decant extract into 15ml glass c-tube
7. Dry down on water bath to 1.0 ml
8. Transfer to GC vial inject 2 uls into GC/NPD or GC/MS

Schemes for the extraction methods. The top scheme succinctly delineates the DPX extraction method. The bottom scheme describes the steps used for the Micro Luke Method.

Instrumentation.

An Agilent 6890N with ECD and NPD detectors was used with dual simultaneous injection auto sampler 7683B. The columns were DB 608 (30 m, 0.32 mm, 0.25um) for ECD and DB 35MS (30 m, 0.32 mm, 0.25 um) and NPD. Injection volumes were1 uL. Oven Temp: 80C for 1min, 30C/min to 190C, hold 1 min, 6C/min to 280 C, hold for 3.3 min; total runtime was 24 min.

The GC-MS used for confirmations was a Perkin-Elmer Clarus 500 with PE built-in Autosampler. The column used was a PE elite 5MS (30m, 0.25 mm, 0.25um). He flow was 1.05 mL/min. Oven Temp: 80C for 1 min, 30C/min to 190C, hold 1 min, 6C/min to 280 C, hold 3.3 min; total runtime was 24 min. SIM methods were used for each compound of interest based on the screening results from ECD/NPD data.

Materials.

The DPX-RP tips were purchased from DPX Labs, LLC (Columbia, SC). The Lever Extractor was also purchased from DPX Labs.

Figure 1. Picture of the DPX extraction method using the Lever Extractor, which is processing 12 fruit and vegetable samples simultaneously.

Figure 2. Pictures of the Modified Luke Extraction Method using liquid-liquid extractions. The time-consuming column cleanup is not necessary for the highly selective GC-NPD analysis of the polar pesticides.

Results and Discussion

Our research using DPX-RP tips has shown that high recoveries are obtained for the analysis of nonpolar pesticides. This is not surprising, as the concept of using reverse phase mechanisms takes advantage of removing and concentrating hydrophobic compounds from aqueous solutions. As shown in Fig. 3, the recoveries of organochlorine pesticides are very high with the DPX method.

For organophosphate pesticides, recoveries were high for most of the compounds as shown in Fig. 4. However, a few pesticides gave poor recoveries. Dichlorvos had recoveries of approximately 50% and mevinphose was only approximately 20%.

A closer examination of the recoveries indicates that it is possible to predict the recovery based on the polarity of the compound. The logP value, which is used to define the polarity of the compound, of 2 or greater give high recoveries using DPX-RP. Figure 5 shows a plot of % recovery vs. logP for the organophosphorous pesticides.

Fortunately, these polar compounds are known to be readily detected by NPD from the Modified Luke Method. By "miniaturizing" this method (Micro Luke), we combine DPX-Micro Luke to have comprehensive analysis of pesticides in fruit and vegetables. A direct comparison of the DPX-Micro Luke Method with the traditional Modified Luke Method is shown for incurred samples from representative weeks of analyses in Fig. 6.

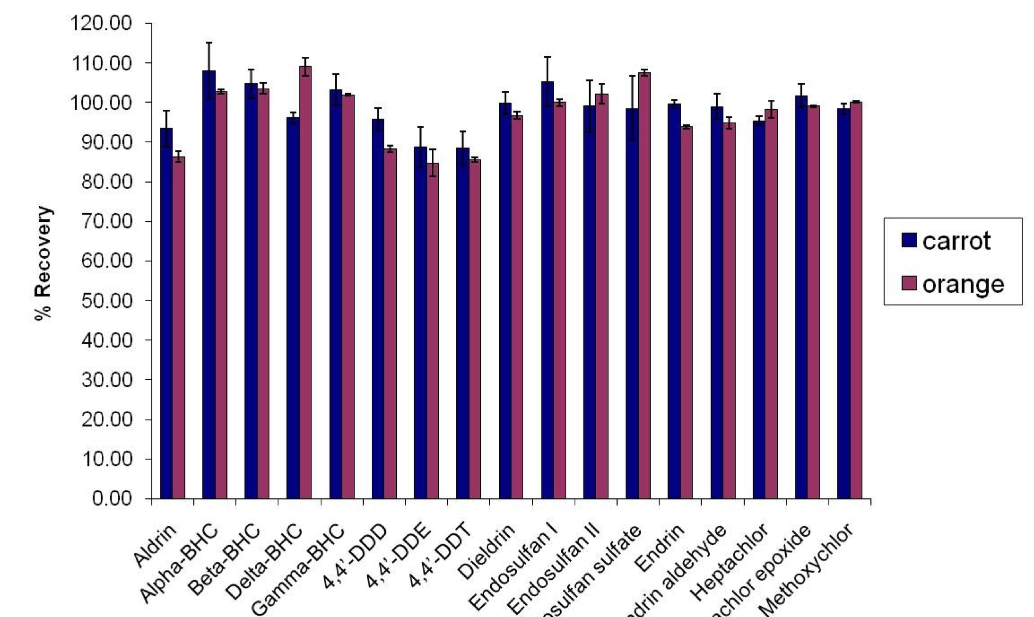

Figure 3. Recoveries of organochlorine pesticides extracted by DPX in carrot and orange sample matrices.

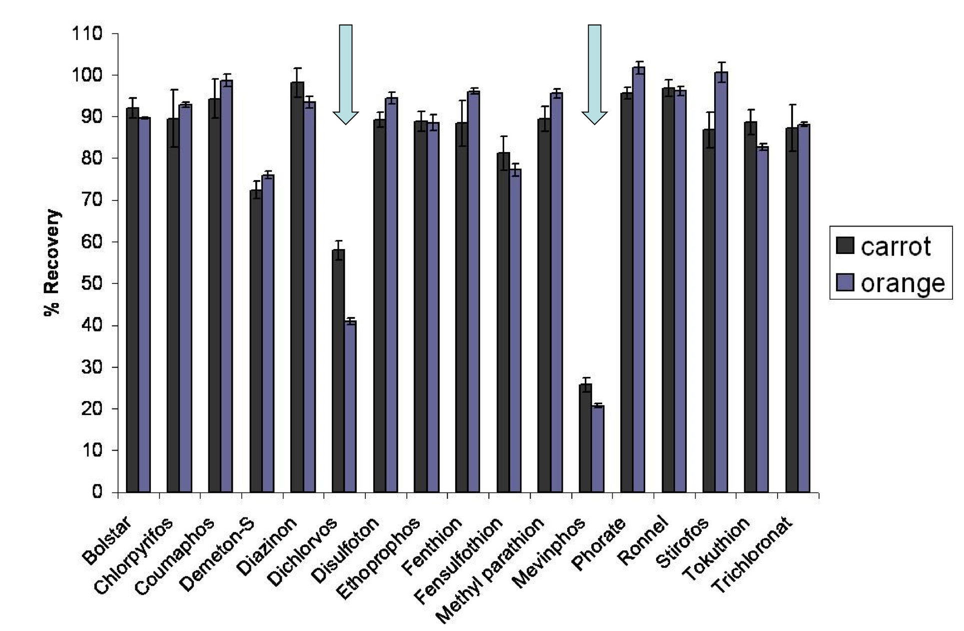

Figure 4. Recoveries of organophosphate pesticides extracted by DPX in carrot and orange sample matrices. The arrows identify compounds with low recoveries.

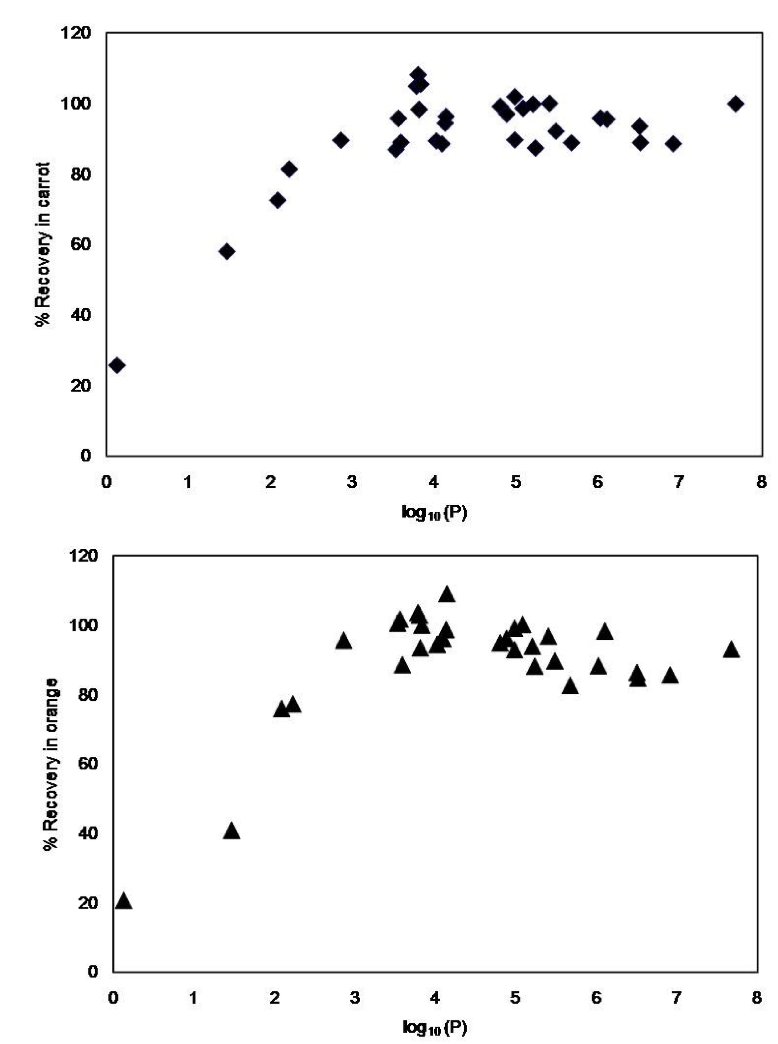

Figure 5. Recoveries of organophosphate pesticides extracted by DPX in carrot (top) and orange (bottom) sample matrices are plotted vs. logP values. Low recoveries are found with polar compounds with logP values less than app. 1.8.

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX/Micro Luke (PPM)	COMPOUND	EPA TOLERANCE (PPM)
62931	Celery	ECD	4.202	5.329	Dicloran	15.0
66560	Tomatoes	ECD	0.473	0.437	Bifenthrin	0.2
	"	ECD	0.183	0.191	Endo I	2.0
	"	ECD	0.110	0.131	Endo II	2.0
	"	ECD	0.010	0.005	Endo Sulfate	2.0
66561	Spinach	ECD	2.792	2.572	Permethrin c & t	20.0
66563	Apple Slices	NPD	0.493	0.732	DPA	10.0
	(NPD macro vs micro Luke)	NPD	0.564	0.788	Thiabendazole	10.0
66564	Blueberries	ECD	0.079	0.071	Esfenvalerate	3.0
	"	ECD	0.075	0.122	Malathion	8.0
		NPD	0.136	0.188	Malathion	8.0
66560	Tomato-SPK @ 0.5 ppm L. Cyhalothrin	ECD	0.393	0.370	L. Cyhalothrin	
	Tomato-SPK @ 1.0 ppm DEF	ECD	0.638	0.648	DEF	
	"	NPD	0.760	1.068	DEF	

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX/Micro Luke (PPM)	COMPOUND	EPA TOLERANCE (PPM)
60081	Gala Apples	ECD	0.0095	0.0129	Dursban	1.5
	(NPD macro vs micro-Luke)	NPD	0.0274	0.0471	Dursban	1.5
	"	NPD	0.7869	1.1844	Thiabendazole	10.0
	"	NPD	NR	0.3652	Azinphos - methyl	2.0
		ECD	NR	0.2243	Azinphos - methyl	2.0
66251	Peaches	ECD	0.2464	0.4411	Phosmet	10.0
	(NPD macro vs. micro Luke)	NPD	0.0758	0.0858	Phosmet	10.0
66638	Snap Beans	ECD	0.1232	0.158	Cypermethrin c & t	0.5
60082	Cukes-SPK @ 2.0 ppm	ECD	1.396	1.5526	Cypermethrin c & t	
	Cukes-SPK @ 2.0 ppm	NPD	0.4318	2.0617	Demeton	
	(NPD - macro vs. micro Luke)					

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX/Micro Luke (PPM)	COMPOUND	EPA TOLERANCE (PPM)
65871	spiked Tomatoes @					
	1.0 ppm Captan	ECD	1.15	0.85	Captan	
	0.05 ppm Chlorothanonil	ECD	0.01	0.03	Chlorothalonil	
	5.0 ppm Acephate	NPD	2.25	2.46	Acephate	
	4.0 ppm Thiabendazole	NPD	5.56	6.99	Thiabendazole	
	(NPD macro vs micro Luke)					

Figure 6. Representative comparisons of results for DPX-Micro Luke vs. the original Modified Luke Method for incurred samples. In most instances, the DPX with Micro Luke Method provided better results for incurred samples.

Conclusions

The DPX-Micro Luke Method has been shown to be a viable method for comprehensive analysis of pesticides in fruit and vegetables. Most importantly, this new methodology results in higher throughput (twice the number of samples) and lower costs (approximately half) for this analysis. These conclusions are depicted in the tables below.

LUKE METHOD

PRODUCT	AMOUNT	COST
Hexane	990 mL @ 0.0091/mL	$9.00
Sodium Chloride	60 gm @ 0.00714/gm	$0.43
Methanol (Wash)	1000 mL @ 0.0036/mL	$3.60
Methylene Chloride	1200 mL @ 0.008/mL	$9.60
Sodium Sulfate	1500 gm @ 0.0109/gm	$16.35
Pet Ether	300 mL @ 0.0142/mL	$4.26
Acetone for Blending	1260 mL @ 0.0092/mL	$11.59
Ethyl Ether	810 mL @ 0.0225/mL	$18.22
GC Vials	24 Vials @ 0.16/Vial	$3.84
GC Caps	24 Caps @ 0.18/Cap	$4.32
Filter Paper	6 Filters @ 0.1961/Filter	$1.18
Printer Paper	96 Sheets @ 0.00654/Sheet	$0.63
Ziplock Sample Bags	6 Bags @ 0.5638/Bag	$3.39
DisposableTransfer Pipets	24 Pipets @ 0.0307/Pipet	$0.74
Florisil	30gms @0.125/gm	$3.75

TOTAL FOR 6 SAMPLES: $90.90

PREP & EXTRACTION TIME: 7.0 HRS
TIME TO RUN SAMPLES ON GC: 6.0 HRS
TIME TO IDENTIFY, QUANTIFY, RECORD RESULTS & REPORT: 4.0 HRS
TOTAL TIME 17.0 HRS

DPX METHOD PLUS MICROLUKE

Using 5 mL RP Tips

PRODUCT	AMOUNT	COST
Hexane	3 mL @ 0.0091/mL	$0.03
Sodium Chloride	30 gm @ 0.00714/gm	$0.22
Methanol (Wash)	500 mL @ 0.0036/mL	$1.80
Methylene Chloride	45 mL @ 0.008/mL	$0.36
Sodium Sulfate	87 gm @ 0.0109/gm	$0.95
Pet Ether	15 mL @ 0.0142/mL	$0.21
Acetone for Blending	1200 mL @ 0.0092/mL	$11.04
Ethyl Acetate	3 mL @ 0.0062/mL	$0.02
GC Vials	12 Vials @ 0.16/Vial	$1.92
GC Caps	12 Caps @ 0.18/Cap	$2.16
Filter Paper	6 Filters @ 0.1961/Filter	$1.18
Printer Paper	36 Sheets @ 0.00654/Sheet	$0.24
Ziplock Sample Bags	6 Bags @ 0.5638/Bag	$3.39
Pasteur Pipets	18 Pipets @ 0.20/Pipet	$3.60
Glass Vial Inserts	6 Inserts @ 0.16/Insert	$0.96
Disposable Culture Tubes	12 Tubes @ 0.09716/Tube	$1.17
DPX-RP 5mL	6 Tips @ $3.00/Tip	$18.00

TOTAL FOR 6 SAMPLES: $47.25

PREP & EXTRACTION TIME: 2.0 HRS
TIME TO RUN SAMPLES ON GC: 2.5 HRS
TIME TO IDENTIFY, QUANTIFY, RECORD RESULTS & REPORT: 4.0 HRS
TOTAL TIME 8.5 HRS