DPX与Micro Luke在杀虫剂残留检测中的应用

Sherry T. Garris and Chanin Craft
South Carolina Dept. of Agriculture, Columbia, SC, USA
Hongxia Guan, William E. Brewer, and Stephen L. Morgan
Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC, USA

摘要

本文描述了一种改良的应用DPX与Micro Luke萃取极性和非极性杀虫剂的方法。这种方法的优势在于选用了针对气相色谱（GC）的不同检测器，如氮-磷检测器（NPD）和电子俘获检测器（ECD）。另外，在这种方法中，可与GC-MS联用（或HPLC-MS联用）。

首先用丙酮进行萃取。一部分丙酮萃取混合液会用液-液快速萃取方法萃取极性杀虫剂，如有机磷酸酯类杀虫剂和有机氮类杀虫剂。另一部分丙酮萃取混合液使用DPX tips进行反相纯化。对分析有机氯杀虫剂的GC-ECD Luke方法来说，不需要使用费时的柱相层析。对大部分的蔬果，DPX纯化可使背景干扰降低。事实上，使用DPX纯化获得的ECD图谱与用Luke方法的结果不相上下。

本研究比较了改良的Micro Luke与DPX结合的方法，及标准的改良Luke方法（柱层析）。DPX方法提高了非极性杀虫剂的回收率，而且样品回收体积相对增加两倍，降低了分析的成本。

Key Words

Gas Chromatography; Sample Preparation; DPX; Pesticides

Introduction

在过去的四年，南卡罗莱纳农业局（SCDA）一直在使用一种新的快速的针对杀虫剂筛选生产和基质的DPX的方法。我们分析比较了DPX、Luke及QuEChERS三种方法。

QuEChERS对大多数杀虫剂的回收率都较好，但其萃取物的杂质很高。对多种混合样品来说，其背景干扰太高而影响鉴别样品中的杀虫剂种类。

DPX对非极性和弱极性杀虫剂来说效果非常好。然而，对个别极性杀虫剂（如乙酰甲胺磷或噻苯咪唑类）来说其回收率较低。

我们研发了一种Micro Luke与DPX结合的方法，同时能萃取极性杀虫剂和非极性杀虫剂，因而代替了费时的柱层析纯化。对不确定的杀虫剂来说（如乙酰甲胺磷类），还会使用传统的液-液萃取和NPD分析。

这些研究的成果非常理想， SCDA化学分析实验室一直在使用这种Micro Luke与DPX结合的方法。由于经费问题，SCDA对每个样品可以做两次而成本减半，同时可以检测更多的化合物。另一个优点是用丙酮代替乙腈，尤其是在乙腈不足或乙腈成本太高的情况下。

Experimental

DPX操作方法——ECD/MSD方法
1.取2.5 mL丙酮萃取混合物并置于一个玻璃检测管中；
2.加入6 mL去离子水和2.5 mL饱和氯化钠；
3.抽入约3.5 mL以上的液体至DPX-RP-5枪头中，抽入约10 mL空气并混合；
4.停留30秒至1分钟，随后推出至废液瓶中；
5.重复至少两次以上，直至所有溶液均抽入至同一个枪头中；
6.在枪头顶端加入1 mL去离子水并推出至废液瓶中；
7.在枪头顶端加入0.7 mL正己烷乙酸乙酯（Hexane-Ethyl acetate）（1+1, v/v），洗脱至GC管中，将上层液体转移至注射管中，并注射进样1 μL。



图1：Lever Extractor与DPX萃取图示，可同时操作12种果蔬样品。

Micro Luke操作方法——NPD/MSD方法
1.取2.5 mL丙酮萃取混合物至50 mL Teflon C-tube w/cap管中；
2.加入2.5 mL饱和氯化钠；
3.加入2.5 mL石油醚；
4.加入7.5 mL二氯甲烷，晃动-放气-振荡20秒；
5.加入约14.5 g硫酸钠，晃动-放气-振荡10秒；
6.离心5-10分钟，将萃取物倒入至15 mL玻璃c-tube中；
7.放置于水浴中干燥至1 mL；
8.移至GC管中，注射进样2 μL于GC/NPD 或 GC/MS。



图2：改良的Luke萃取与液-液纯化图示，极性杀虫剂的GC-NPD分析中不需要费时的柱纯化。

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.

仪器设备：
1.Agilent 6890N + ECD/NPD检测器+双重同时样品自动注射器7683B；
2.ECD检测器的柱子为DB 608（30 m, 0.32 mm, 0.25 μm）；NPD检测器的柱子为DB 35MS（30 m, 0.32 mm, 0.25 μm）；
3.注射体积为1 μL；
4.加热温度：80℃ 1分钟，每分钟30℃直至190℃停留1分钟，每分钟6℃直至280℃，停留3.3分钟，总长24分钟；
5.SIM方法用来分析目的化合物。
材料：
DPX-RP枪头及Lever Extractor均购于DPX公司。

Results and Discussion

结果显示，应用DPX-RP纯化非极性杀虫剂获得较高的回收率。这与预期结果相符，因为使用反相纯化在去除杂质及浓缩疏水化合物中有独特的优势。如图3所示，DPX方法获得的有机氯杀虫剂的回收率非常高。

对有机磷酸酯类杀虫剂，大多数的化合物回收率较高（图4），但一部分回收率较低。敌敌畏回收率为50%，速灭磷仅有20%。

对回收率的进一步分析显示，可以用化合物的极性来预测回收率。化合物的极性用logP值来显示，DPX-RP的logP值为2或更高，说明回收率较高。图5为有机磷杀虫剂回收率百分比与logP值的关系。

幸运的是，应用改良的Luke方法，这些极性化合物易于被NPD检测。为了使这种方法更简便（Micro Luke），我们将DPX与Micro Luke结合以期广泛应用于果蔬中杀虫剂的检测。图6为DPX-Micro Luke方法与传统的Luke方法的比较结果。

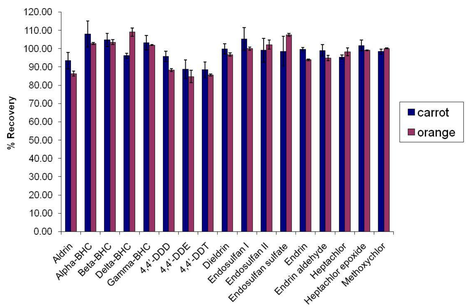


图3：胡萝卜和橙子样品中有机氯杀虫剂的回收率（DPX方法）。

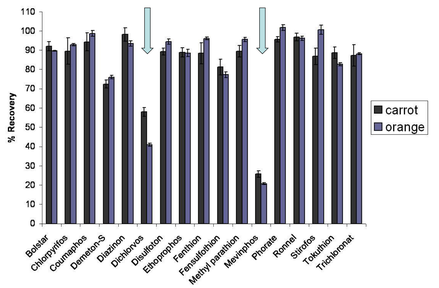


图4：胡萝卜和橙子样品中有机磷酸酯杀虫剂的回收率（DPX方法）。箭头指示的是低回收率化合物。

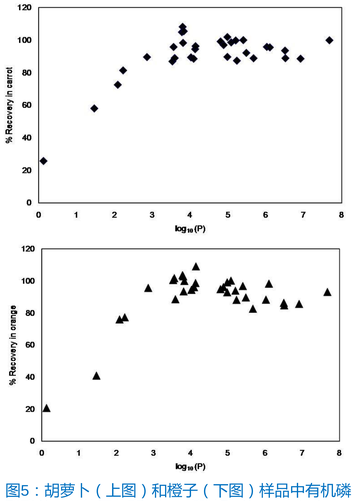


图5：胡萝卜（上图）和橙子（下图）样品中有机磷酸酯杀虫剂的回收率与logP值关系（DPX方法）。logP值小于1.8为低回收率的极性化合物。

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 macro vs micro Luke)	NPD	5.56	6.99	Thiabendazole	

图6：DPX-Micro Luke与传统改良的Luke方法的比较。DPX-Micro Luke方法对于大部分样本都有较好的结果。

Conclusions

本研究结果证实了DPX-Micro Luke方法可广泛应用于果蔬中杀虫剂的检测。更重要的是，这种新的方法使样品回收体积增加两倍，并且降低约两倍的成本。具体数据请见下表。

LUKE METHOD

PRODUCT	AMOUNT	COST
Hexane	990 mL @ 0.0091/mL	$9.00
Sodium Chloride	60 gm @ 0.0071/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 @ 0.0065/Sheet	$0.63
Ziplock Sample Bags	6 Bags @ 0.5638/Bag	$3.39
DisposableTransfer Pipets	24 Pipets @ 0.030/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 @ 0.0071/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 @ 0.0065/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

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

Sherry T. Garris and Chanin Craft
South Carolina Dept. of Agriculture, Columbia, SC, USA
Hongxia Guan, William E. Brewer, and Stephen L. Morgan
Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC, USA

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.

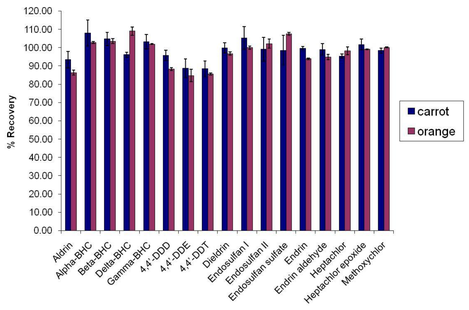


Figure 3. Recoveries of organochlorine pesticides extracted by DPX in carrot and orange sample matrices.

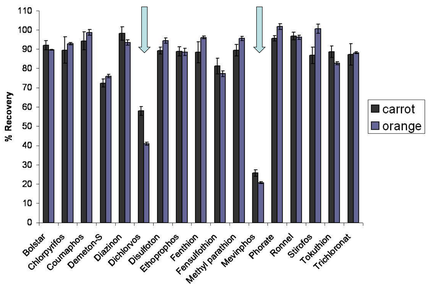


Figure 4. Recoveries of organophosphate pesticides extracted by DPX in carrot and orange sample matrices. The arrows identify compounds with low recoveries.

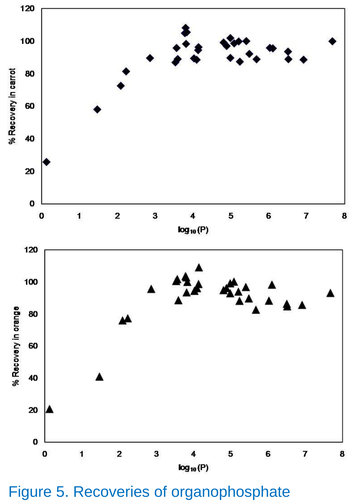


Figure 5. Recoveries of organophosphate pesticides extracted by DPX in carrot (top) and orange (bottom) sample matrices are plotted vs. logP values. Low recoveries are found with polar compounds with logP values less than app. 1.8.

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX/Micro Luke (PPM)	COMPOUND	EPA TOLERANCE (PPM)
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	"	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
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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 @ 0.0065/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: $90.99

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 @ 0.0065/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