

Multi-Residue Pesticide Analysis on Incurred Produce Samples Using Variations of Disposable Pipette Extraction

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DPX Movie of Dye Extraction

Demonstration of Dispersive SPE in a Tip



Outline of Presentation

- I. AOAC QuEChERS Study—DPX comparison
- II. Multi-Residue Study Using GC/NPD and GC/ECD
- III. DPX-Q—QuEChERS in a Tip
- IV. Future Studies: Automation (GERSTEL MPS-2)

I. DPX in QuEChERS AOAC Study

Extract in acetonitrile following QuEChERS procedure

15 g sample plus 15 mL ACN

Add salt (MgSO_4 and NaCl)

Shake, centrifuge

I. DPX in QuEChERS AOAC Study

DPX-RP (nonpolar compounds):

Take 1 mL and add 2.4 mL H₂O plus 0.8 mL sat'd NaCl

Perform DPX extraction (app. 3-4 minutes)

Water wash step; elution with hexanes/ethyl acetate

Inject into **GC-ECD** (*no solvent evaporation*)

I. DPX in QuEChERS AOAC Study

DPX-CU (polar compounds):

Take 1 mL ACN solution

Aspirate in-and-out of DPX cleanup (polyamino) tip

Solvent exchange into “acetone”

Inject into **GC-NPD**

(Acetone should have been replaced with toluene.)

I. DPX Results

DPX-RP

Orange (GC-ECD)	type	ECD	%RSD
HCB	OC	49.9	3.2
chlorothalonil	OC	63.9	6.8
chloryriphos	OC/OP	58.6	5.2
endosulfan-I	OC	62.1	6.9
p,p'-DDE	OC	52.4	6.0
ethion	OP	64.2	4.4
endofulfan-II	OC	63.0	6.7
endofulfan-SO4	OC	67.9	7.9
phosmet	OP	66.4	6.4
azinphos-methyl	OP	111.2	8.4
trans-permethrin	OC	105.9	12.6

DPX-Polyamino (cleanup)

Orange (GC-NPD)	type	NPD	RSD
methamidophos	OP	69.7	8.2
Acephate	OP	80.3	12.8
Chloryriphos	OC/OP	88.7	6.1
TPP (int std)	OP	89.6	7.1

Lower recoveries with elutions from the “bottom**” (similar results with spinach).**
 Elute from the top and obtain app. 100% recoveries of nonpolar pesticides (see charts in next slides).

I. DPX Results

DPX-RP

Carrots (GC-ECD)	type	ECD	%RSD
HCB	OC	91.7	4.5
chlorothalonil	OC	111.9	4.9
chloryriphos	OC/OP	103.2	3.5
endosulfan-I	OC	99.2	4.4
p,p'-DDE	OC	89	1
endofulfan-II	OC	99.9	2.9
ethion	OP	98.7	3.6
endofulfan-SO4	OC	103.8	1.7
phosmet	OP	108	0.2
azinphos-methyl	OP	107.1	1.5
trans-permethrin	OC	81	8.3

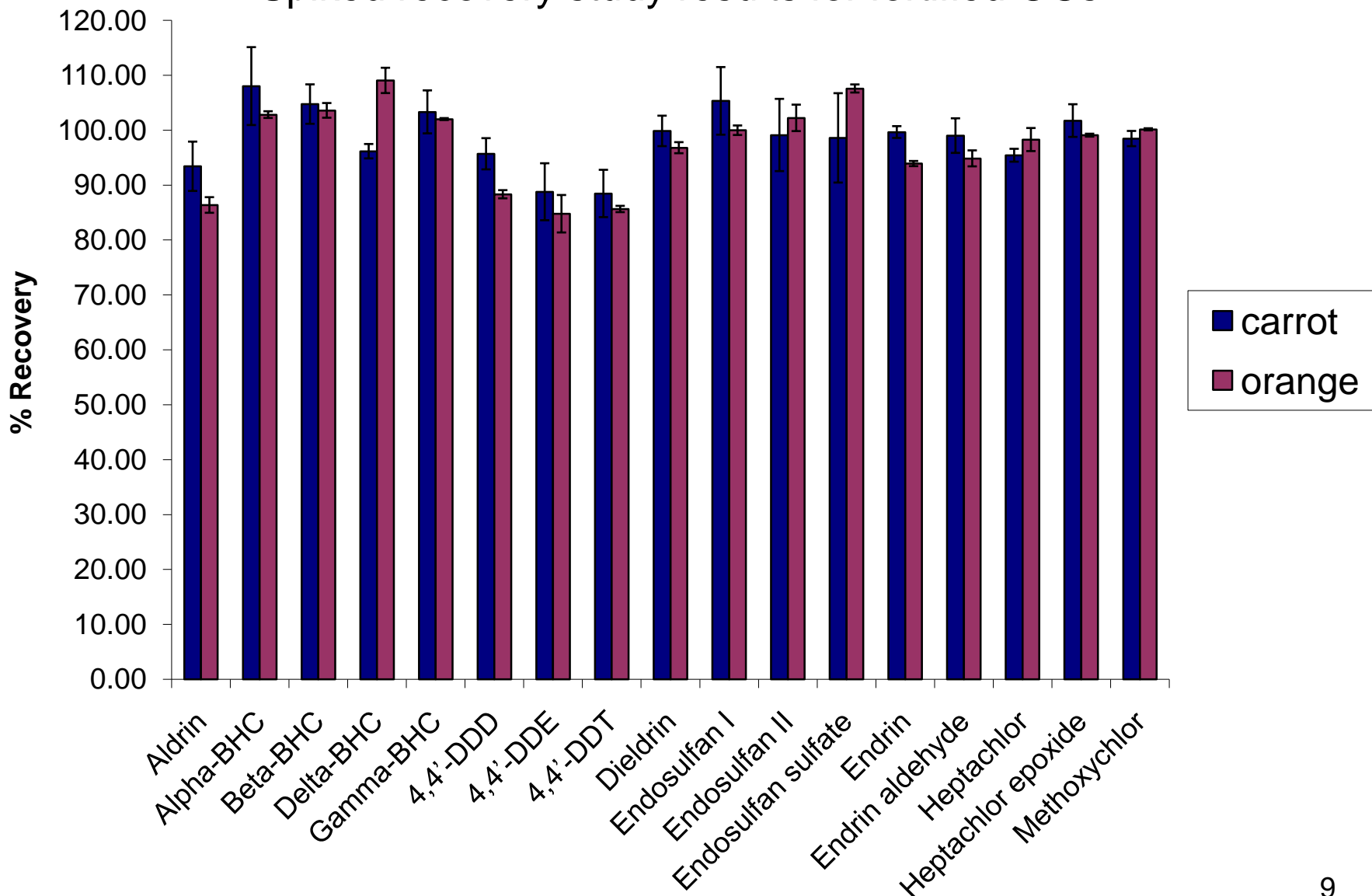
DPX-Polyamino (cleanup)

Carrots (GC-NPD)	type	NPD	%RSD
methamidophos	OP	58.9	7.3
acephate	OP	58.3	16.9
chloryriphos	OC/OP	77.4	3.9
TPP (int std)	OP	78.8	3.7

Top elution provides highest recoveries! Polyamino cleanup (to remove fatty acids) can be improved with additional solvent or by replacing with PSA. Polyamino has more active sites and acts as a stronger anion exchange sorbent..—better for removing fatty acids (gastric contents, tissue, grain, etc.).

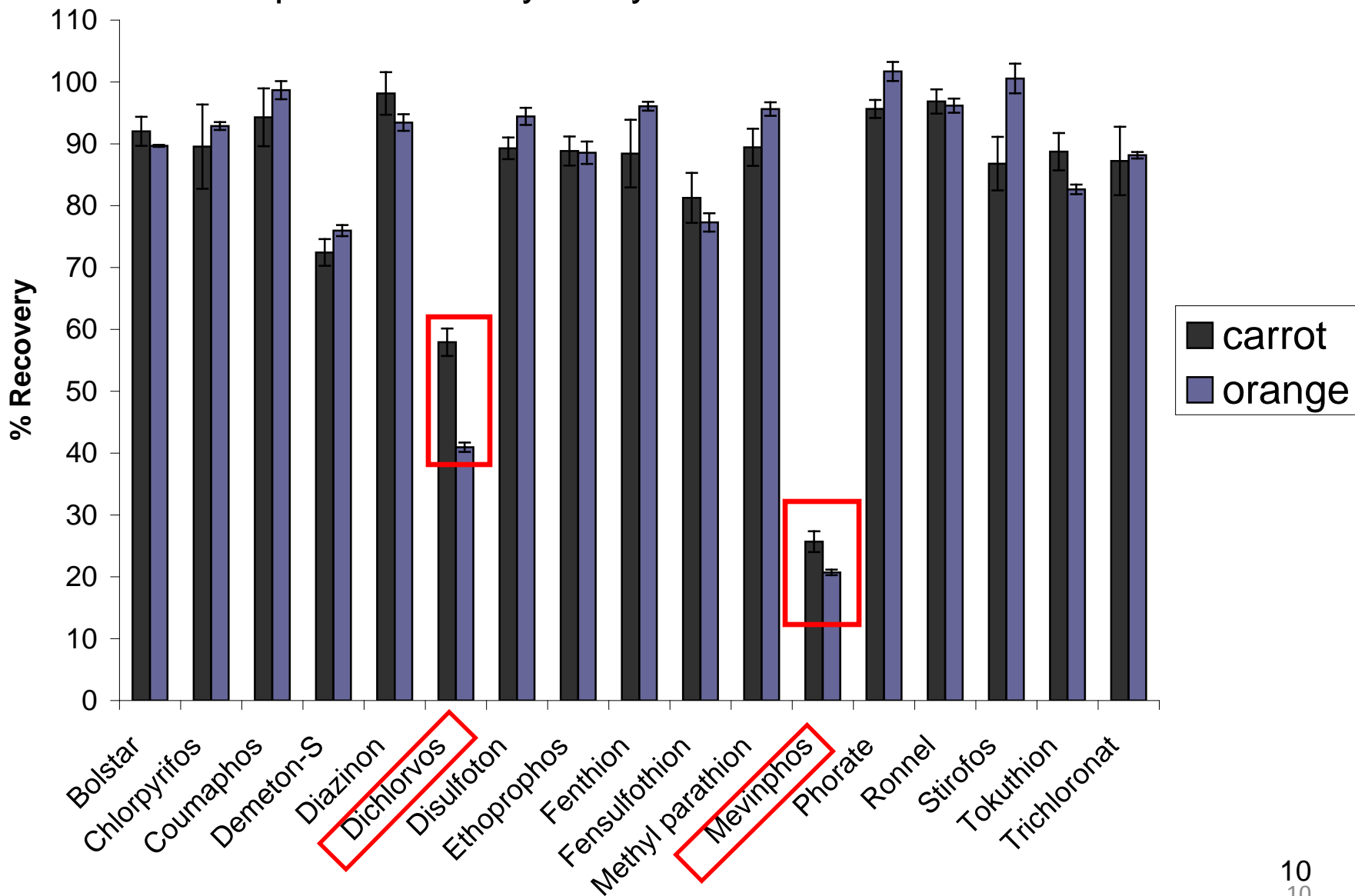
Results and discussion

Spiked recovery study results for fortified OCs



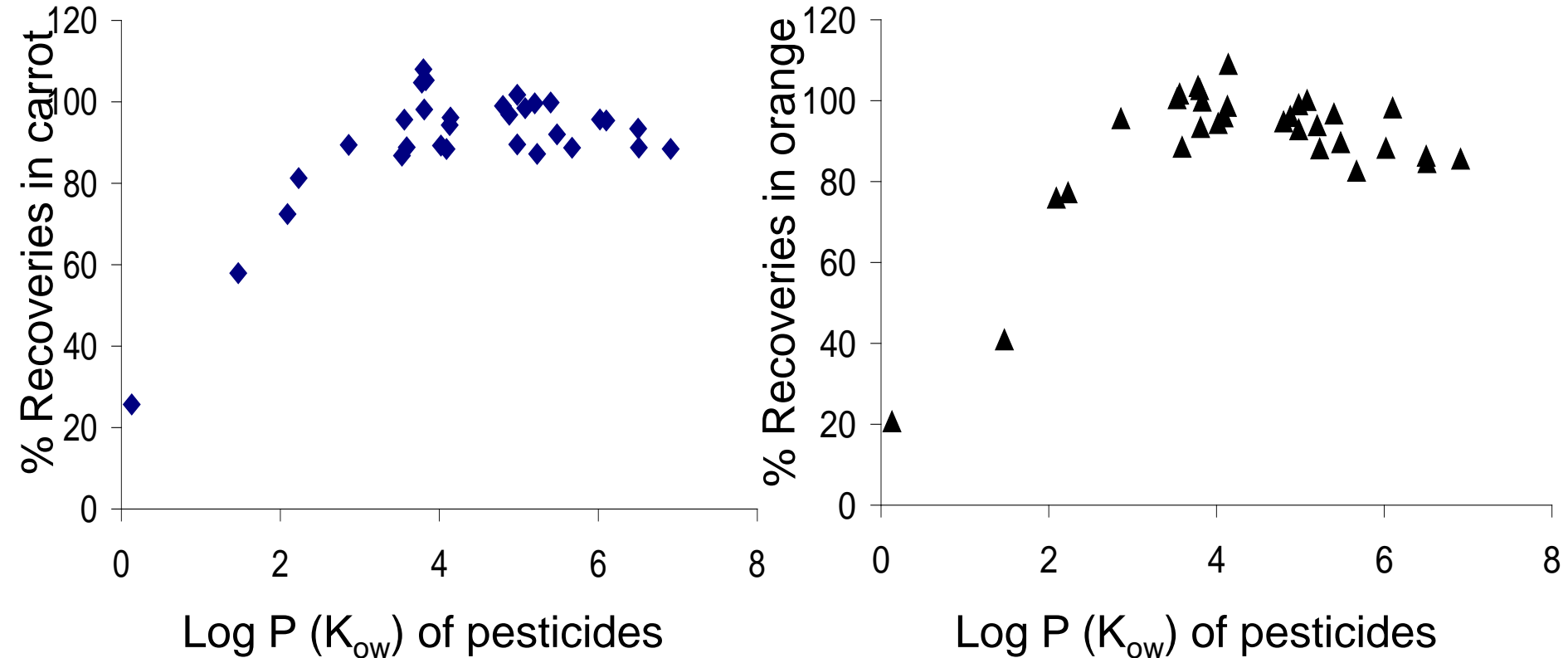
Results and discussion

Spiked recovery study results for fortified OPs



Results and discussion

Hydrophobicity and recovery

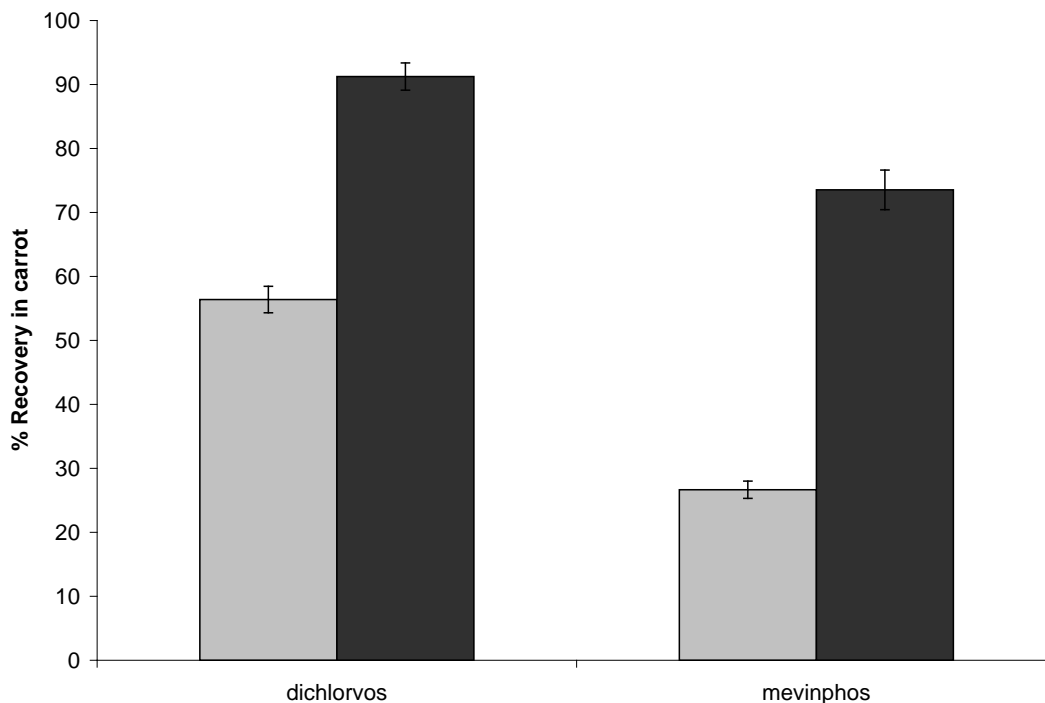


It is possible to estimate the recovery using the log P (K_{ow}) value of the pesticide.

Results and discussion

What did we do to improve recoveries for polar OPs ?

Adding large volume of DI H₂O to “breakthrough solution”



□ OP extraction using 2.4 mL DI H₂O, (n=4)

■ Extraction of “breakthrough” solution by adding extra 8 mL DI H₂O, (n=4)

Linearity and LOD for studied OCs

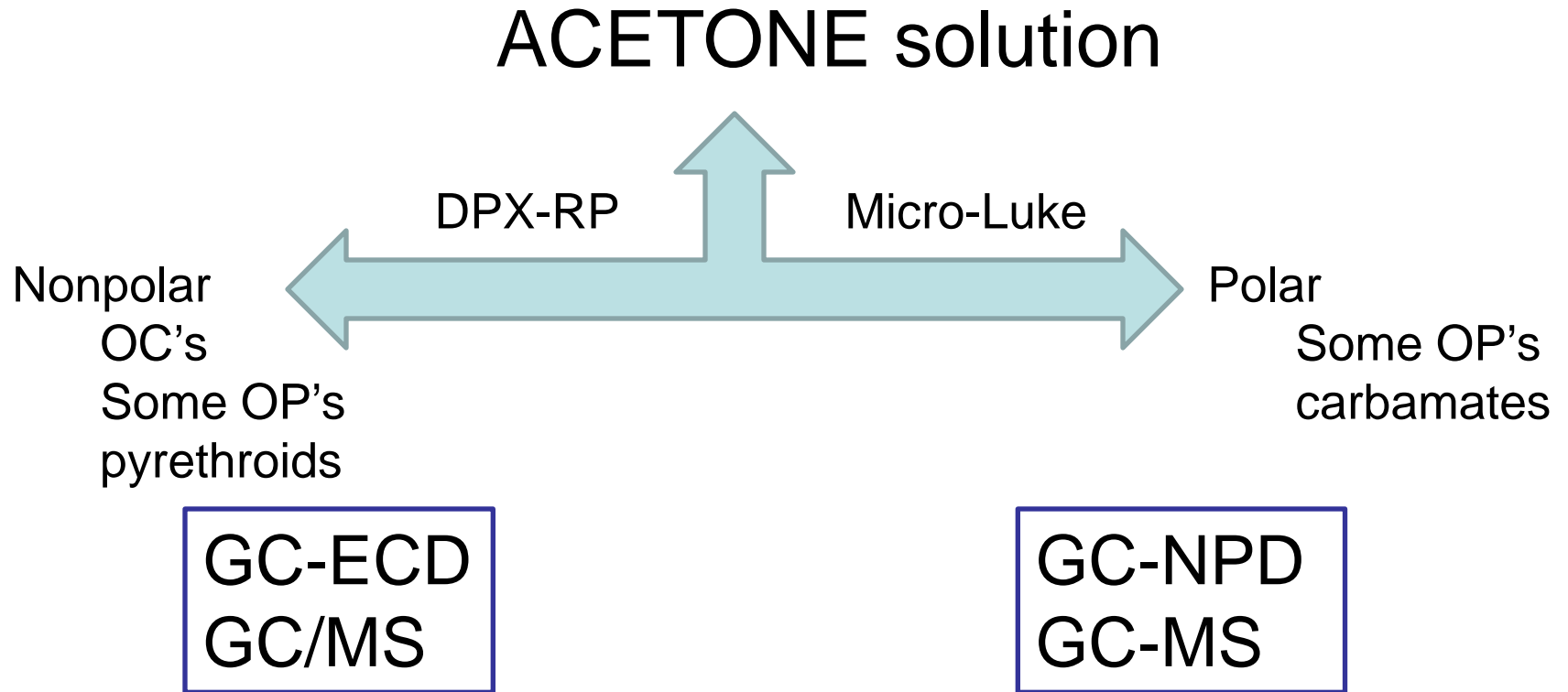
Pesticide	r^2		LOD (ppm)	
	carrot	orange	carrot	orange
Aldrin	0.9979	0.9984	0.0172	0.0308
Alpha-BHC	0.9988	0.9988	0.0072	0.0216
Beta-BHC	0.9991	0.9986	0.0069	0.0234
Delta-BHC	0.9990	0.9983	0.0329	0.0333
Gamma-BHC	0.9991	0.9987	0.0100	0.0119
4,4'-DDD	0.9986	0.9968	0.0039	0.0741
4,4'-DDE	0.9955	0.9981	0.0141	0.0324
4,4'-DDT	0.9974	0.9957	0.0220	0.0629
Dieldrin	0.9991	0.9990	0.0170	0.0388
Endosulfan I	0.9990	0.9987	0.0099	0.0334
Endosulfan II	0.9974	0.9984	0.0136	0.0592
Endosulfan sulfate	0.9990	0.9954	0.0182	0.0806
Endrin	0.9992	0.9986	0.0246	0.0624
Endrin aldehyde	0.9980	0.9987	0.0481	0.0272
Heptachlor	0.9984	0.9984	0.0116	0.0460
Heptachlor epoxide	0.9978	0.9986	0.0224	0.0221
Methoxychlor	0.9982	0.9965	0.0051	0.0506

r^2 : coefficient of determination, LOD: limit of detection.

Linearity and LOD for studied OPs

Pesticide	r ²		LOD (ppm)	
	carrot	orange	carrot	orange
Bolstar	0.9977	0.9983	0.0061	0.0277
Chlorpyrifos	0.9990	0.9996	0.0134	0.0374
Coumaphos	0.9976	0.9984	0.0066	0.0576
Demeton-S	0.9972	0.9995	0.0201	0.0201
Diazinon	0.9996	0.9997	0.0331	0.0213
Dichlorvos	0.9984	0.9967	0.0120	0.0153
Disulfoton	0.9995	0.9978	0.0101	0.0128
Ethoprophos	0.9997	0.9992	0.0143	0.0246
Fenthion	0.9990	0.9995	0.0051	0.0227
Fensulfothion	0.9977	0.9990	0.0093	0.0270
Methyl parathion	0.9991	0.9978	0.0139	0.0187
Mevinphos	0.9945	0.9989	0.0228	0.0352
Phorate	0.9996	0.9990	0.0049	0.0208
Ronnel	0.9993	0.9995	0.0058	0.0213
Stirofos	0.9992	0.9956	0.0224	0.0718
Tokuthion	0.9988	0.9983	0.0129	0.0355
Trichloronat	0.9994	0.9993	0.0189	0.0141

II. DPX v. Luke Method



II. DPX v. Luke Method

- Blend 100 grams of produce with 200 mls of Acetone
- Filter Extract through glass fiber filter paper

- NPD detector—no solvent exchange necessary (acetonitrile)

II. DPX procedure ---- ECD method

1. Place 2.5 mls of acetone extract into a glass test tube

2. Add 6.0 mls of D.I. water and 2.0 mls of saturated NaCl

3. Draw in about 3.5 mls of total solution to a DPX-RP 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 and draw in all the solution to 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

Inject "top" organic layer (small volume of immiscible water at bottom).

II. MicroLUKE procedure ---- NPD method

1. Place 2.5 ml of acetone extract into a glass test tube



2. Add 1.0 ml of saturated NaCl, vortex mix 10 sec.



3. Add 2.5 ml of petroleum ether, vortex mix 10 sec.



4. Add 7.5 mls of methylene chloride, vortex mix 10 sec.



5. Stand for 5 min to let the two layers separated clearly



6. Upper layer drain through about 4.0 g of anhydrous sodium sulfate



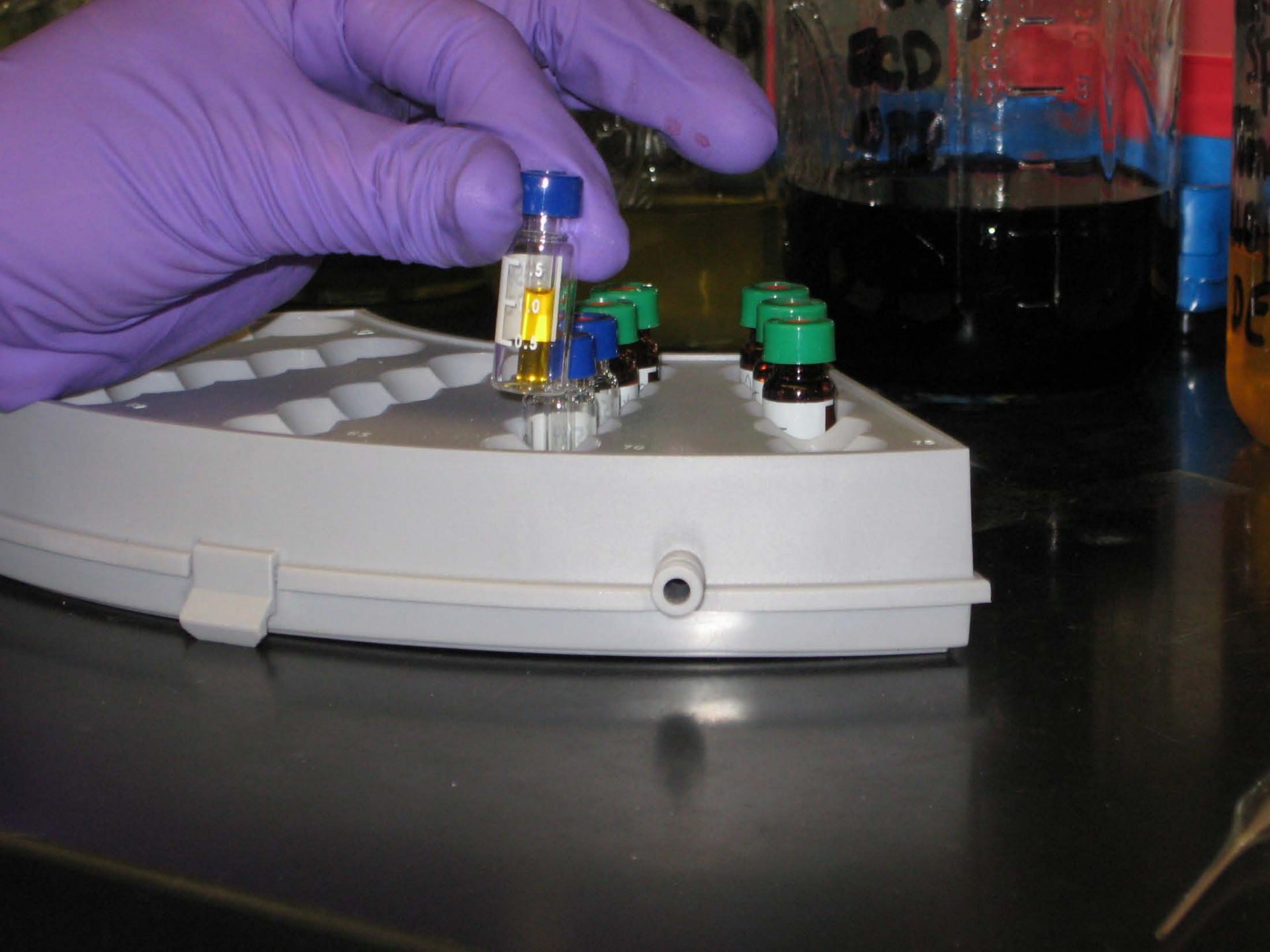
7. Wash the sodium sulfate with 1 ml of methylene chloride



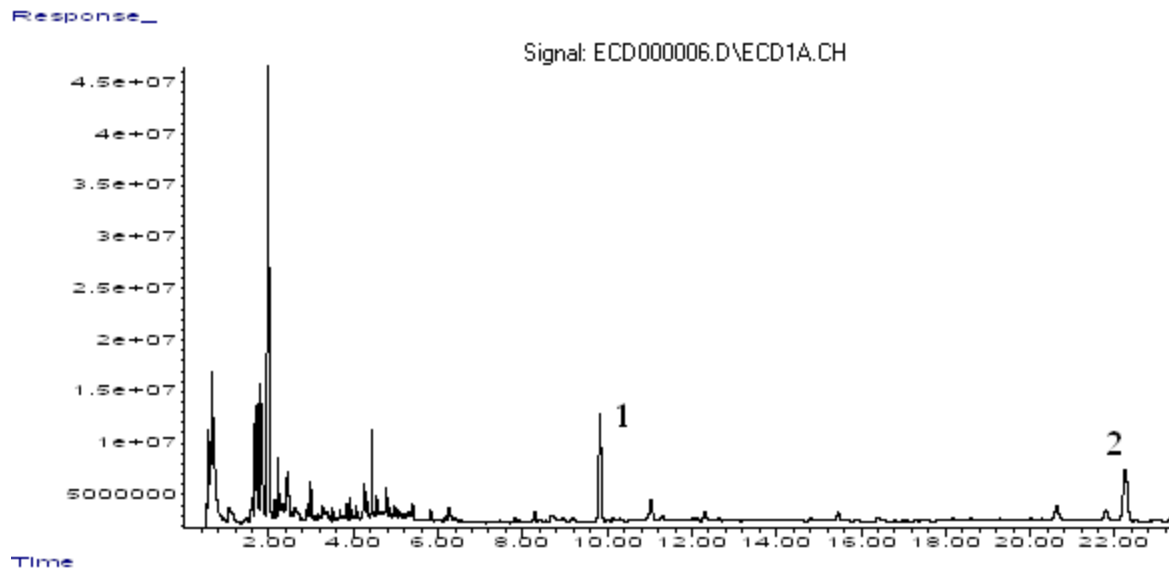
8. Transfer all the extract to C-tube and dry down to 0.5 ml







II. GC/ECD data using DPX-RP

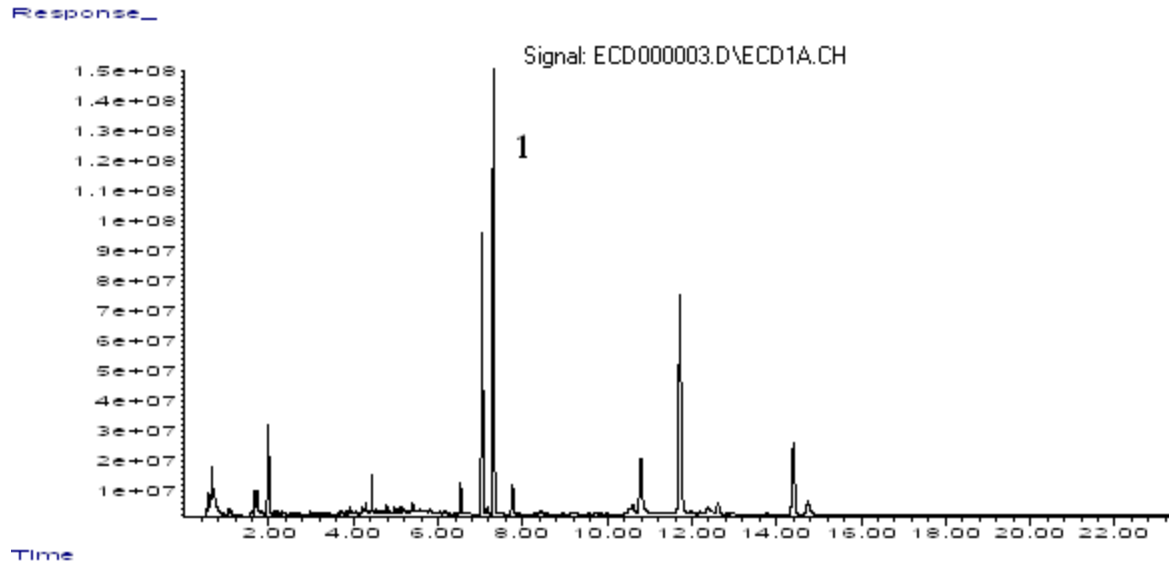


Blueberries

1-malathion

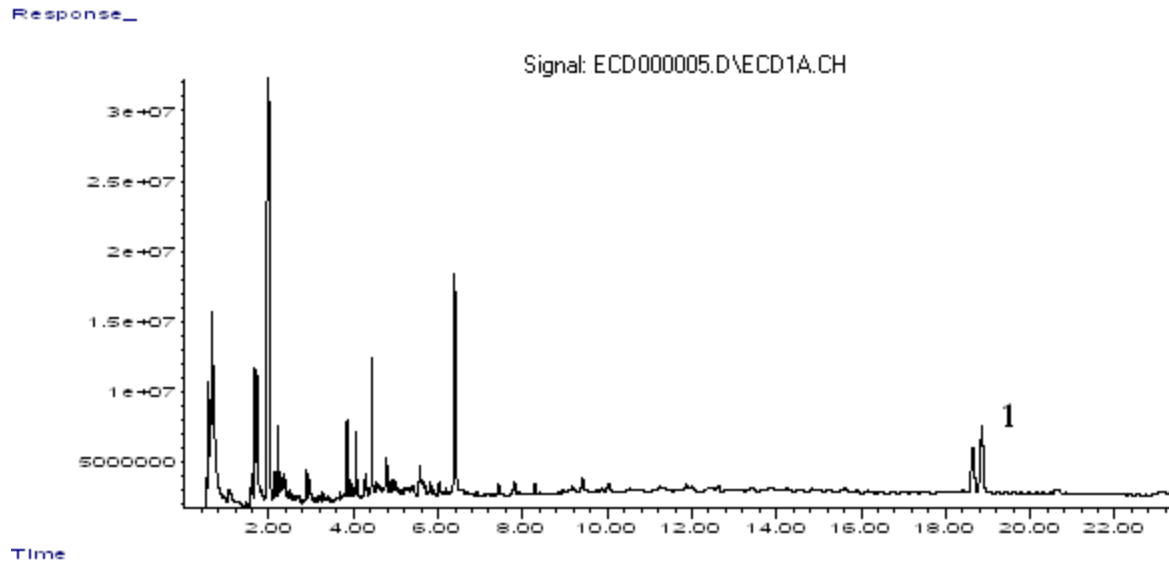
2-esfenvalerate

II. GC/ECD data using DPX-RP



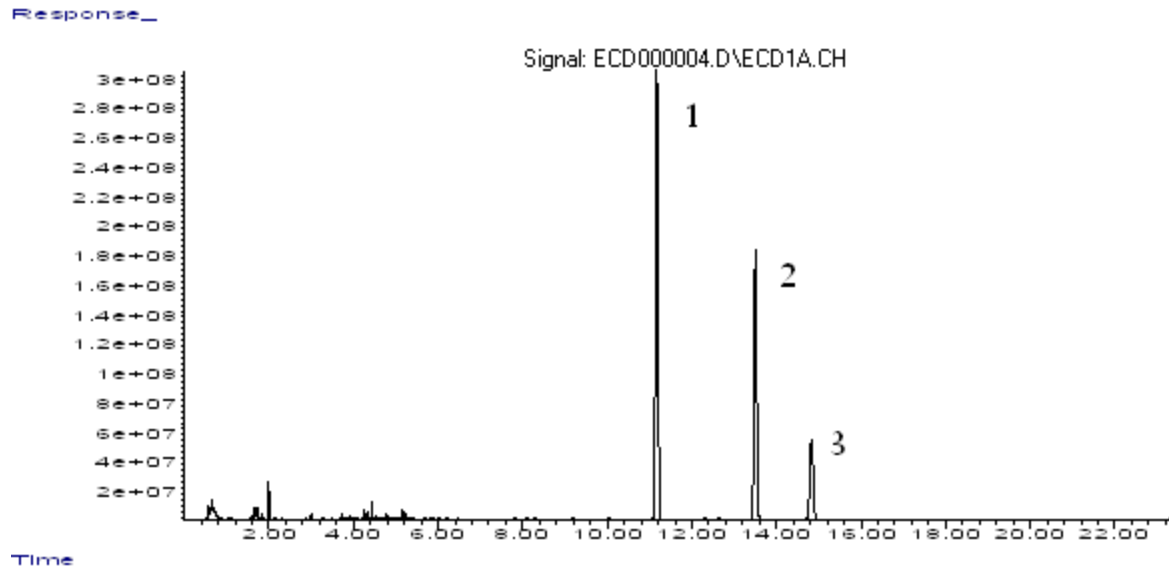
Celery
1-dicloran

II. GC/ECD data using DPX-RP



Spinach
1- permethrin

II. GC/ECD data using DPX-RP



Tomatoes

1-endosulfan I

2-endosulfan II

3-endosulfan sulfate and bifenthrin

II. Results of DPX-RP and Micro-Luke (week 2)

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX (PPM)	COMPOUND	EPA TOLERANCE (PPM)
65885	SPK Strawberries @ 0.05	ECD	0.022	0.160	Chlorothalonil	NT
	SPK Strawberries @ 20.0	NPD	20.72	27.85	Carbaryl	10.0
	Strawberries - Incurred	ECD	0.03	0.06	Captan	25.0
	"	ECD	0.27	0.41	Bifenthrin	3.0
65497	Apple Bites	ECD	0.22	0.29	Captan	25.0
	"	NPD	0.55	0.34	DPA	10.0
65498	Red Apples	ECD	0.42	0.45	Captan	25.0
	"	ECD	0.02	0.03	Endosulfan	2.0
	"	NPD	0.30	1.64	DPA	10.0
65881	Celery	ECD	0.28	0.38	Dicloran	15.0
65883	Pears	ECD	0.75	0.99	Captan	25.0
	"	ECD	0.03	0.05	Bifenthrin	0.5
	"	ECD	0.03	0.04	L. Cyhalothrin	2.5
62875	Red Grapes	ECD	0.14	0.20	Dursban	1.5
65579	Yellow Squash	ECD	0.0152	0.0238	Endo I	2.0
	"	ECD	NR	0.0036	Endo II	2.0
	(Endo Sulfate and Bifenthrin co-elute on DPX)	ECD	0.0106	0.0327	Endo Sulfate	2.0
		ECD	0.1900	NR	Bifenthrin	0.4
	"	ECD	0.100	NR	Chlorothalonil	5.0

II. Results of DPX-RP and Micro-Luke

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX (PPM)	COMPOUND	EPA TOLERANCE (PPM)
47323	Strawberries	ECD	4.80	8.39	Captan	25.0
	"	ECD	0.0066	0.0142	Endo I	2.0
	"	ECD	0.0315	0.0666	Endo II	2.0
	"	ECD	0.1037	0.0224	Endo Sulfate	2.0
64288	Tomatoes	ECD	0.02	0.06	Chlorothalonil	5.0
65521	Bell Peppers	ECD	0.06	0.08	Chlorpyrifos (Dursban)	1.0
	"	NPD	0.14	0.21	Chlorpyrifos (Dursban)	1.0
65525	Grape Tomatoes	ECD	0.06	0.22	Chlorothalonil	5.0
66006	Strawberries	ECD	0.60	0.85	Captan	25.0
64287	SPK - Orange @ 0.05 ppm	ECD	0.0082	0.0314	Chlorothalonil	
	SPK - Orange @ 5.0 ppm	NPD	2.37	0.26	Acephate	

Micro-Luke method was not optimized “yet” for analysis of polar compounds (acephate).

Week 3

II. Results of DPX-RP and Micro-Luke

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX (PPM)	COMPOUND	EPA TOLERANCE (PPM)
66126	Blueberries	ECD	0.08	0.15	Esfenvalerate	3.0
66129	Baking Potatoes	ECD	0.006	0.005	p,p, DDE	NT - A.L. 1.0
63645	Peaches	ECD	0.17	0.32	Phosmet	10.0
	"	NPD	0.09	0.06	L/L Phosmet - no clean-up	10.0
65198	Apricots	ECD	0.09	0.20	Phosmet	5.0
	"	NPD	0.03	0.03	L/L Phosmet - no clean-up	5.0
63643	Yellow Squash spiked @ 20.0 ppm	ECD	13.96	20.07	Chlorpropham	
	"	NPD	13.68	21.22	Chlorpropham	
	(Started NPD macro vs micro Luke	Liquid / Liquid micro Luke Extraction - no clean-up for NPD				
63643	Yellow Squash spiked @ 10.0 ppm	NPD	10.90	16.03	Carbofuran	

Week 4

II. Results of DPX-RP and Micro-Luke

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX (PPM)	COMPOUND	EPA TOLERANCE (PPM)
61824	Cucumbers spiked @ 1.0 ppm	ECD	0.733	0.975	Chlorpyrifos ethyl (Dursban)	
	(NPD macro vs micro Luke)	NPD	0.079	1.070	Chlorpyrifos ethyl (Dursban)	
61824	Cucumbers spiked @ 1.0 ppm	ECD	0.740	0.855	Cyfluthrin	

II. Results of DPX-RP and Micro-Luke

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX (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	

Week 6

II. Results of DPX-RP and Micro-Luke

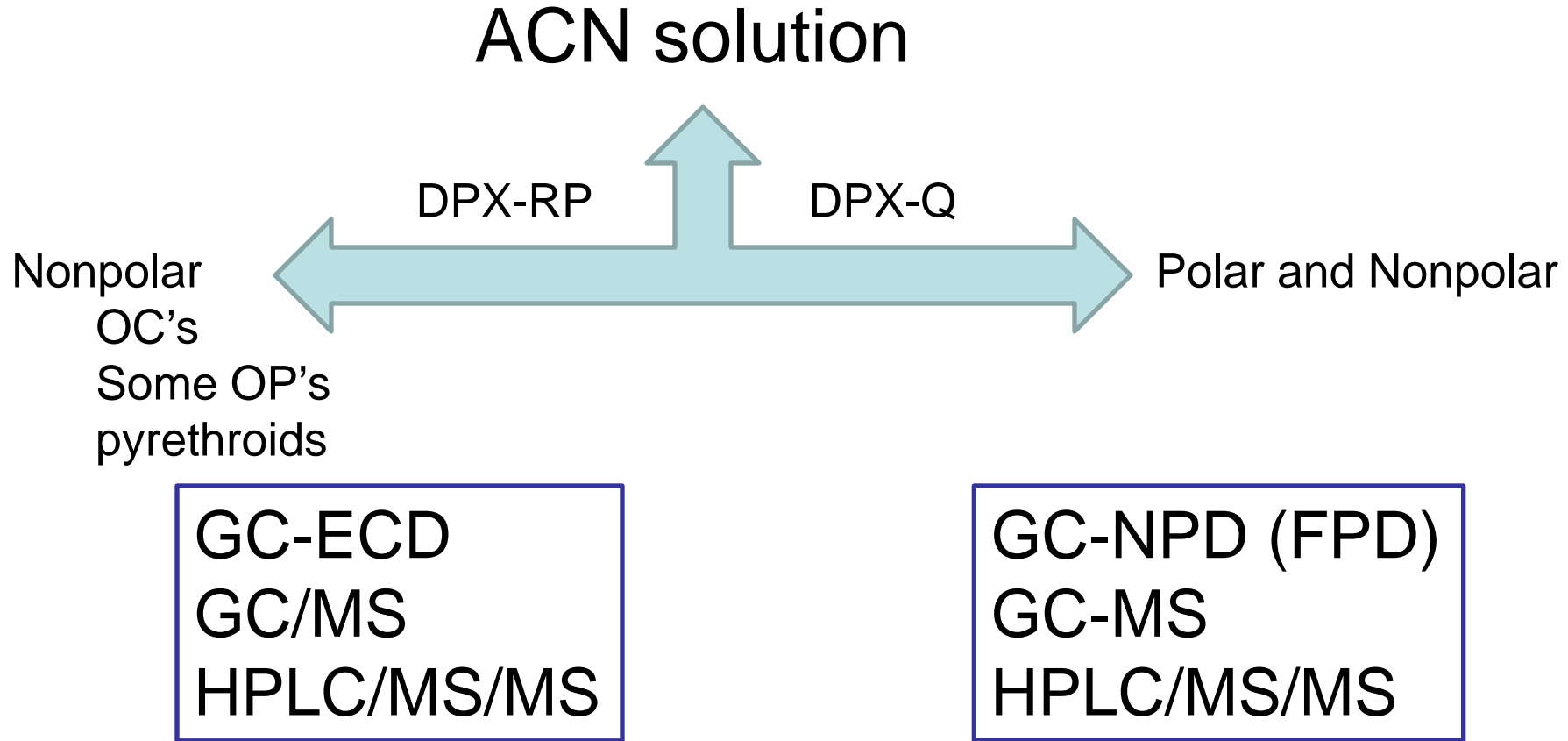
SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX (PPM)	COMPOUND	EPA TOLERANCE (PPM)
60081	Gala Apples	ECD	0.0095	0.0129	Dursban	1.5
	(NPD macro vs micro-Luke)	NPD	0.0274	0.0471	Dursban	1.5
	"	NPD	0.7869	1.1844	Thiabendazole	10.0
	"	NPD	NR	0.3652	Azinphos - methyl	2.0
		ECD	NR	0.2243	Azinphos - methyl	2.0
66251	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)					

Week 7

II. Results of DPX-RP and Micro-Luke

SAMPLE ID	MATRIX	DETECTOR	LUKE (PPM)	DPX (PPM)	COMPOUND	EPA TOLERANCE (PPM)
65871	spiked Tomatoes @					
	1.0 ppm Captan	ECD	1.15	0.85	Captan	
	0.05 ppm Chlorothalonil	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)					

III. DPX-Q scheme



III. DPX-Q QuEChERS Tips

DPX-Q (polar compounds):

1. Take 2.5 mL ACN solution from original pre-sample preparation
2. Extract in and out of “DPX-Q” 2-3 times
Rapidly remove water and salts (app. 1 minute)
3. Inject into **HPLC/MS/MS or GC/MS**
OR solvent exchange and inject into GC-NPD
Takes several minutes (app. 20 min) to solvent exchange

III. DPX-Q for polar OP's (GC/MS)

Pesticides	Vegetable/fruit	Spiked conc. (ppm)	DPX method	% recovery (n=4)	%RSD (n=4)
Acephate	Spinach	5.0	GCB/MgSO ₄ /PSA ^a	99.88	3.92
	Orange	5.0		109.5	1.38
Dichlorphos	Spinach	0.5	GCB/MgSO ₄ /PSA ^a	91.56	2.09
	Orange	0.5		100.55	2.00
Mevinphos	Spinach	0.5	GCB/MgSO ₄ /PSA ^a	90.38	1.86
	Orange	0.5		94.51	3.79
Methamidophos	Spinach	2.0	GCB/MgSO ₄ /PSA ^a	96.32	9.60
	Orange	2.0		103.06	2.59

Procedures:

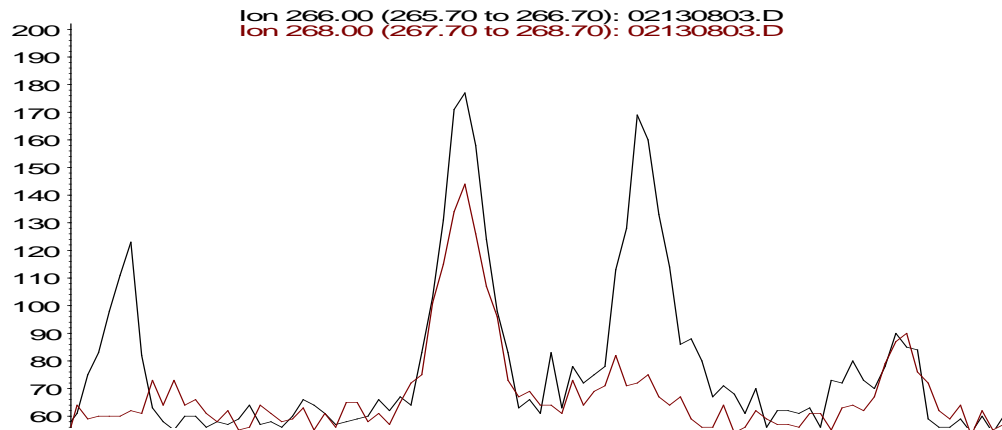
- 1ml acetonitrile solution, add 300µL of toluene (total 1.3 mL sample solution).
- Draw in DPX (150 mg mixed GCB/MgSO₄/PSA) three times from the bottom to remove sample matrix.
- Dispense and collect after cleanup (total about 1 mL).
- Add “external” standard.
- Dry down to 0.5 mL.
- Inject.

III. DPX-RP for captan and chlorothalonil

pesticides	Vegetable/fruit	Spiked conc. (ppm)	DPX method	%recovery	% RSD
Captan	Spinach	2.0	SDVB	104.48	1.19
	Orange	2.0	SDVB	91.81	5.35
chlorothalonil	Spinach	0.5	SDVB	113.57	1.91
	Orange	0.5	SDVB	115.28	9.80

10 ppb chlorothalonil extracted by DPX-RP using GC/MS (SIM)

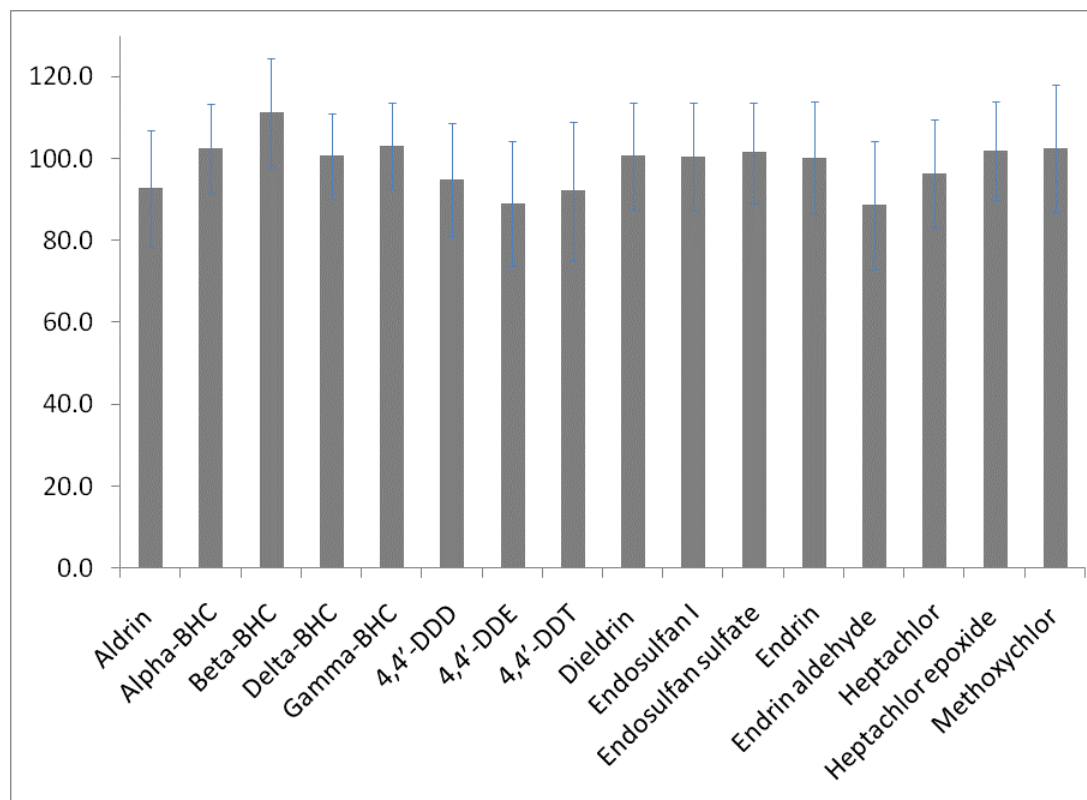
Abundance



IV. DPX -- GERSTELIZED



IV. % Recovery of OCs in carrot extracted using automated DPX with GERSTEL MPS=2



OCs: spiked 0.5ppm in carrot

Conclusions

DPX-RP provided “cleaner” extracts than QuEChERS (modified) for GC-ECD data.

High recoveries were achieved for all of the studied pesticides by combining DPX-RP and DPX-Q, with %RSD lower than 10%. This indicates a good accuracy and precision of the current method.

DPX method is more environmentally friendly.

Average coefficient of determination, $r^2 > 0.995$, indicates a good linearity of the current DPX method.

Current research focuses on full automation of DPX using GERSTEL MPS with:

- Large volume injection (LVI, GERSTEL PTV)
- fast GC (ECD, FPD)
- HPLC/MS/MS

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