



Development and validation of a multi-residue method for neonicotinoid and macrocyclic lactone pesticides in milk and 11 fruit and vegetable commodities by LC-MS/MS

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BACKGROUND

- **The neonicotinoids and the macrocyclic lactones are relatively new classes of insecticides; only recently been registered by EPA.**
- **The Health Effects Division (HED)/ EPA Office of Pesticide Programs (OPP) will assess the risk from dietary exposure to the newly registered pesticides based on real world monitoring data.**
- **The USDA Pesticide Data Program (PDP) laboratories routinely provide needed residue monitoring data to the specific analyte/commodity combinations requested by HED.**




**Target Analyte, Commodity List and Target LOD
“Neonicotinoids and metabolites”**

Neonicotinoid	Commodity	Target LOD
1. Dinotefuran 2. Dinotefuran UF	Grape, lettuce, watermelon, spinach, broccoli	0.05 ppm
3. Thiamethoxam 4. Clothianidin	Apple, broccoli, spinach, tomato	0.005 ppm
5. Imidacloprid 6. Imidacloprid urea 7. Imidacloprid olefin 8. Desnitroimidacloprid olefin 9. Desnitroimidacloprid HCl	Grape, orange, apple, peach, plum, green bean	0.01 ppm
10. Acetamiprid 11. Acetamiprid IM-1-2	Apple, spinach, lettuce	0.05 ppm
12. Thiacloprid	Meat, milk	0.001 ppm
13. Flonicamid	Tomato, lettuce, apple, spinach	0.05 ppm




**Target Analyte, Commodity List and Target LOD
“macrocylic lactones”**

Chemical	Commodity	Target LOD
1. Avermectin B1a	Plum, watermelon, milk, strawberry, squash	0.01 ppb
2. Emamectin B1a	Milk, apple, pear, collard, spinach	0.1 ppb
3. Spinosyn A	Milk, lettuce, orange, collard, spinach	0.05 ppm
4. Spinetoram(XAD-175J)		
5. Milbemycin A4		
6. Moxidectin		
7. Doramectin		
8. Eprinomectin		
9. Ivermectin		



OBJECTIVES

- **Modify/develop a MRM, which can be used by PDP laboratories without major deviations from the current CDFA-MRM.**
- **Achieve the lowest method limits of detection and quantitation possible for the individual target analytes.**
- **Developed rugged and reliable method to obtain residue data at levels low enough to accurately determine the chemical risk from dietary exposure .**
- **Validate the method by the analysis of sufficient number of replicates at three fortification levels (LOQ, 2X LOQ & 10X LOQ) for all commodities.**
- **Recovery goal: 70 – 120% with RSD<20. However, recoveries of 50-150% would be acceptable since the goal of this method is to obtain residue data at the 1 – 10 ppb range.**



Procedure used by PDP-CA (CDFA method)

Homogenize and extract sample by blending

↓ **50g sample, 100 ml ACN, filter**

C₁₈ SPE Cleanup

↓ **pass extract, collect, add 20g NaCl,
refrigerate (about 3 hours)**

Florisil® SPE Cleanup

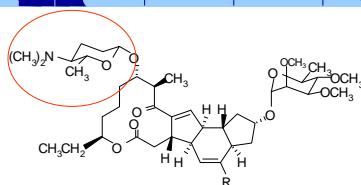
↓ **pass 5 ml, dry, add 1 ml IS in MeOH:H₂O**

Instrument Analysis (LC-MS/MS)



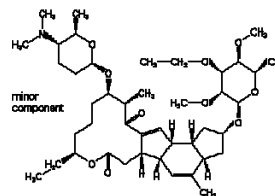
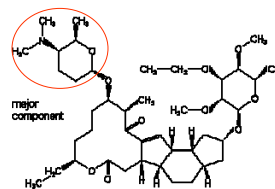
Recoveries of macrocyclic lactones

- Following the CDFA procedure, recoveries of:
 - Spinosyn A was 32%
 - Emamectin B_{1a} was 28%
 - Spinetoram was 30%
- When C-18 & FL SPE cartridges were rinsed with an additional 5 ml of 2% TEA in ACN, recoveries of these compounds increased to ~ 80%

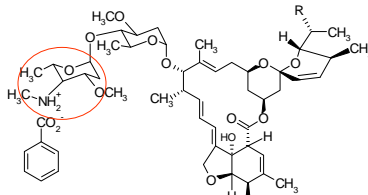


spinosyn A, R = H-

spinosyn D, R = CH₃-



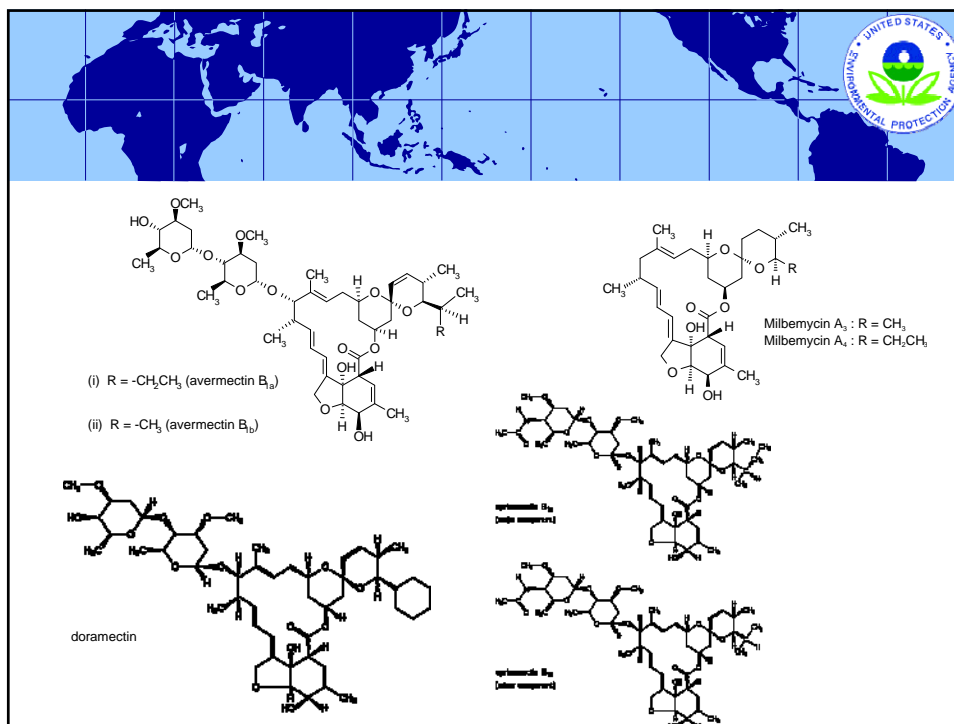

spinetoram



Emamectin benzoate

B_{1a} R = CH₂CH₂-

B_{1b} R = CH₃-

Modified Procedure

Homogenize and extract sample by blending

↓ 50g sample, 100 ml 1% TEA in ACN, filter


C₁₈ SPE Cleanup

↓ pass extract, collect, add 20g NaCl, refrigerate (about 3 hours)

Florisil® SPE Cleanup


↓ pass 5 ml, dry, add 1 ml IS in MeOH:H₂O (1:1)

Instrument Analysis (LC-MS/MS)




LC-MS/MS

- Waters Acquity UPLC/Quattro Premier triple quadrupole mass spectrometer
- Column: Waters X-bridge (150 x 2.1 mm), 3.5 μm particle size Mobile phase: A: (95:5) water: methanol + 10 mM ammonium formate + 0.1% formic acid
B: (95:5) methanol: water + 10 mM ammonium formate + 0.1% formic acid
Gradient from 95% A to 100% B in 20 minutes
- ESI positive ion mode
- Monitored two precursor/product ion transitions for each analyte




Neonicotinoid recoveries

Analyte Neonicotinoid/ Replica	Apple		Orange		Spinach		Bean		W-melon		St-berry	
	10	8	8	8	6	6	2	2	4	4	4	4
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Flonicamid	101.8	9.3	114.3	11.0	102.1	10.1	111.8	17.7	103.4	8.1	108.3	12.4
Thiamethoxam	97.2	9.6	107.5	7.7	99.2	10.3	93.2	18.2	102.3	7.1	105.7	13.4
Imidacloprid	104.8	10.9	113.9	7.5	104.8	10.0	101.6	19.7	109.4	10.0	117.7	14.9
Clothianidin	102.8	9.5	113.2	7.3	103.7	10.8	102.4	16.6	110.3	10.0	116.2	16.9
Acetamiprid	104.0	10.4	114.9	7.6	105.2	10.8	103.4	17.0	107.2	7.4	106.3	16.0
Thiacloprid	103.8	10.5	115.2	13.1	106.7	11.0	103.3	15.9	108.1	7.9	106.9	10.4
Dinotefuran	88.6	9.2	93.6	9.8	85.3	9.0	92.7	11.4	88.3	6.5	85.1	9.6
Analyte Neonicotinoid/ Replica	Plum		Zucchini		Broccoli		Tomato		Grape		Milk	
	4	2	2	2	2	2	4	2	2	2	6	6
Flonicamid	107.5	11.9	91.8	3.5	87.7	14.3	92.9	8.9	116.0	7.8	98.1	0.6
Thiamethoxam	102.1	10.2	91.8	4.1	81.4	7.2	95.6	9.9	117.5	6.5	103.2	0.7
Imidacloprid	126.3	20.9	94.0	4.6	85.8	7.2	97.1	10.0	115.3	8.4	105.1	1.0
Clothianidin	118.9	22.7	97.0	7.6	82.2	5.0	96.4	8.1	110.7	9.3	101.2	0.2
Acetamiprid	108.4	15.1	98.2	4.1	88.3	8.1	95.3	8.7	111.5	7.8	104.7	0.8
Thiacloprid	107.3	10.8	101.4	5.0	88.9	8.2	97.2	8.0	114.7	8.2	108.0	0.7
Dinotefuran	92.5	9.1	80.1	6.0	81.8	7.6	84.4	7.1	93.5	4.2	92.4	0.8



Neonicotinoid metabolite recoveries

Neonicotinoid metabolite	Apple		Orange		Spinach		Bean		W-melon		St-berry	
	10	8	6	2	4	4	4	2	4	4	4	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Dinotefuran UF	52.5	6.6	54.3	5.2	39.9	5.1	57.7	7.4	43.5	6.1	40.0	7.8
Desnitro imidacloprid olefin	46.0	4.2	71.8	30.9	8.5	1.3	11.9	1.6	19.3	13.1	2.1	2.3
Desnitro imidacloprid HCl	6.4	0.7	13.4	11.2	5.6	4.9	25.8	8.8	20.6	18.0		
Imidacloprid olefin	47.1	14.0	85.5	14.6	25.1	8.0	52.2	18.3	49.7	8.0	104.6	13.6
Imidacloprid urea	89.7	8.2	97.4	10.2	86.2	9.3	84.5	16.3	60.7	19.5	95.6	13.5
Acetamiprid IM-1-2	87.5	11.5	89.0	11.9	80.5	10.1	40.1	8.7	78.2	12.0	75.1	21.1
	Plum		Zucchini		Broccoli		Tomato		Grape		Milk	
	4	2	2	4	2	6	4	2	6	4	2	6
Dinotefuran UF	42.8	6.3	44.1	3.8	37.8	3.8	42.2	2.8	31.1	1.7	52.3	2.4
Desnitro imidacloprid olefin	12.1	8.3	3.0	1.6	2.6	1.4	11.9	4.0	2.9	1.4	5.8	3.0
Desnitro imidacloprid HCl	5.9	8.7	6.2	11.2	9.1	4.5	13.3	14.3	10.2	1.9	15.2	2.5
Imidacloprid olefin	75.4	16.5	22.5	4.4	41.1	5.2	59.3	15.4	33.7	7.4	40.6	0.9
Imidacloprid urea	84.3	6.5	79.6	5.3	55.5	6.5	83.8	9.5	84.9	27.2	93.9	0.7
Acetamiprid IM-1-2	87.8	11.1	95.7	11.8	44.9	7.7	103.4	16.0	96.5	10.4	134.0	6.9



Macroyclic lactone recoveries

Macroyclic lactone	Apple		Orange		Spinach		Bean		W-melon		St-berry	
	10	8	6	2	4	4	4	2	4	4	4	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Spinosyn A	93.3	12.9	91.6	20.7	103.2	6.6	84.1	14.0	86.6	4.7	82.1	12.2
Spinetoram	87.7	10.4	81.8	10.5	99.3	7.9	59.2	10.9	68.1	5.8	72.4	17.2
Emamectin Benzoate B _{1a}	102.4	12.3	84.6	8.4	104.3	9.4	97.2	19.5	96.5	5.6	65.9	6.0
Avermectin B _{1a}	102.4	17.3	106.9	13.9	111.5	10.0	115.9	20.3	103.6	14.2	91.5	10.8
Milbemycin A4	90.5	16.1	109.3	15.4	101.1	12.0	96.9	11.4	94.9	8.8	85.9	15.6
Moxidectin	75.8	18.0	101.2	13.9	119.8	20.3	88.3	23.2	81.7	6.2	95.9	19.8
Doramectin	88.9	16.9	102.5	11.3	111.5	14.0	112.0	21.9	87.0	27.5	83.6	15.4
Eprinomectin B _{1a}	94.3	14.3	97.8	15.6	99.1	12.2	116.2	21.0	93.7	20.2	101.2	11.5
Ivermectin H ₂ B _{1a}	80.2	11.5	87.0	15.4	109.2	11.3	99.3	19.8	84.4	13.0	79.8	13.2

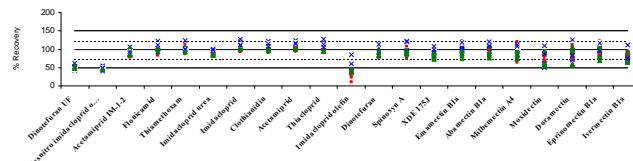


Macrocytic lactone recoveries

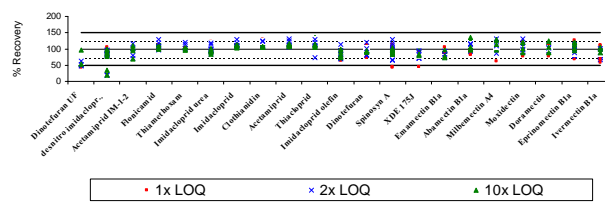
Macrocytic lactone	Plum		Zucchini		Broccoli		Tomato		Grape		Milk	
	4		2		2		4		2		6	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Spinosyn A	91.6	8.6	73.9	3.0	52.8	26.0	79.6	9.9	75.0	13.1	87.1	6.4
Spinetoram	82.5	6.0	58.6	8.4	55.2	3.7	67.4	9.2	76.1	23.8	77.9	2.9
Emamectin Benzoate B _{1a}	86.8	16.8	79.2	11.9	73.1	4.4	93.2	7.9	91.3	8.0	80.2	24.8
Avermectin B _{1a}	103.2	7.6	83.4	13.4	88.0	9.4	99.9	15.4	106.7	20.2	106.7	6.4
Milbemycin A4	94.4	12.9	88.1	11.3	65.0	12.5	90.3	11.4	112.5	15.5	81.7	21.0
Moxidectin	99.1	14.5	82.3	12.8	69.2	8.7	86.4	17.7	106.8	27.6	85.6	20.7
Doramectin	103.9	11.4	74.7	15.3	78.8	7.3	81.3	42.1	87.0	24.8	107.6	4.7
Eprinomectin B _{1a}	104.7	15.0	80.4	11.8	83.3	8.7	94.4	33.9	109.2	10.5	110.3	16.4
Ivermectin H ₂ B _{1a}	94.6	14.0	73.4	11.5	77.7	10.2	90.2	10.7	111.3	18.7	97.1	5.7

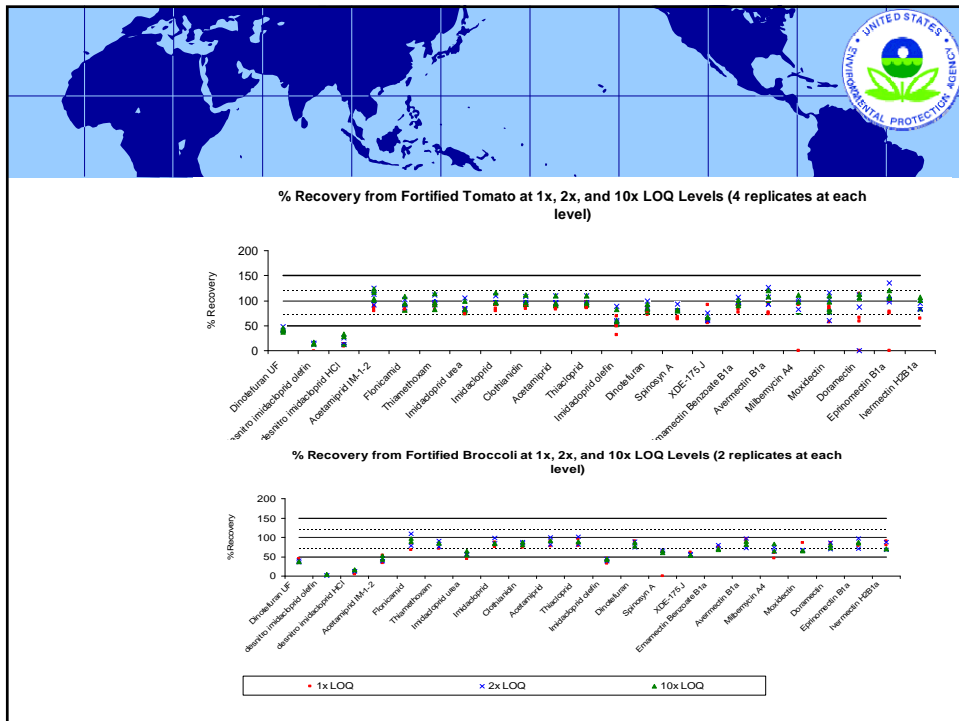
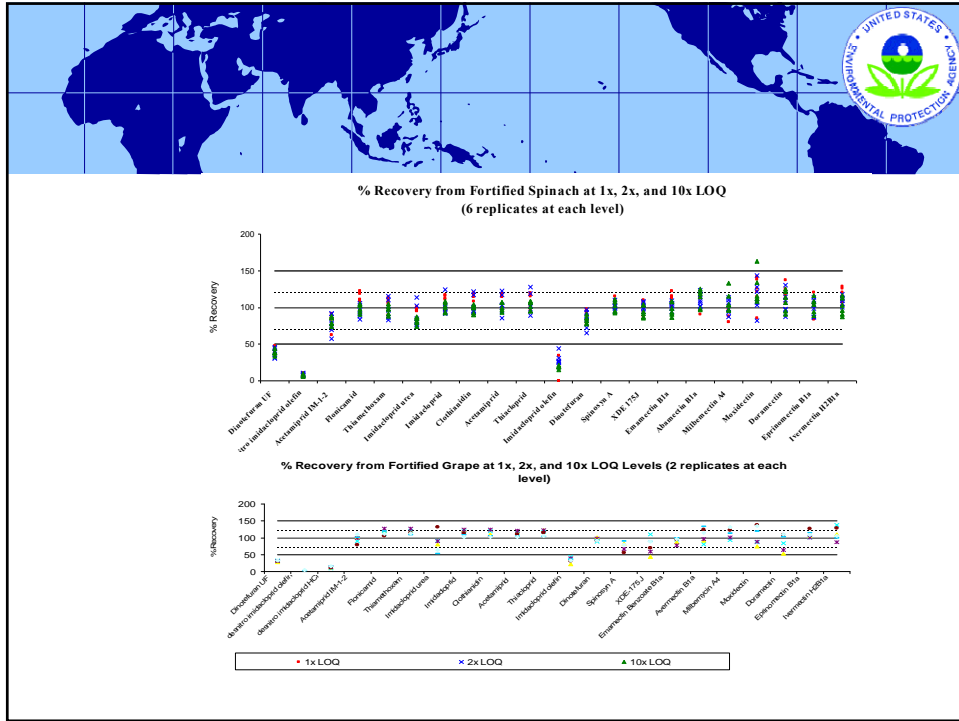


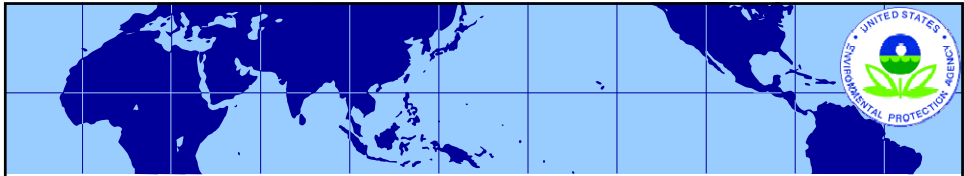
% Recovery from Fortified Apple at 1x, 2x, and 10x LOQ
(10 replicates at each level)



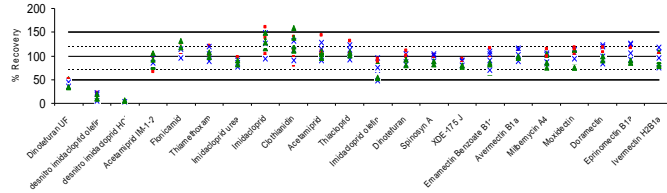
% Recovery from Fortified Orange at 1x, 2x, and 10x LOQ
(8 replicates at each level)



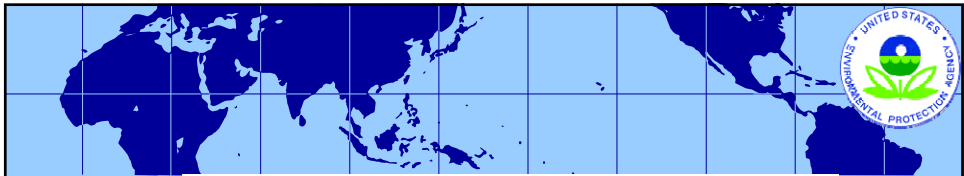
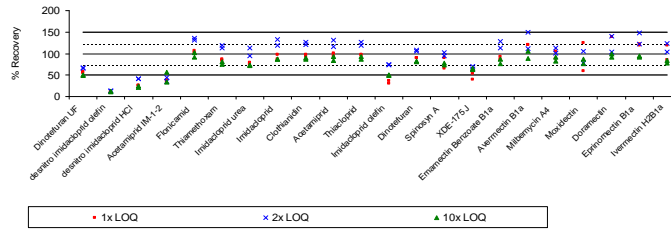




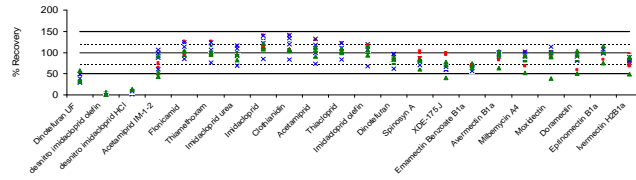
% Recovery from Fortified Plum at 1x, 2x, and 10x LOQ Levels (4 replicates at each level)



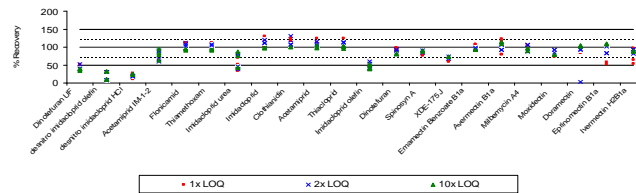
% Recovery from Fortified Green Bean at 1x, 2x, and 10x LOQ Levels (2 replicates at each level)

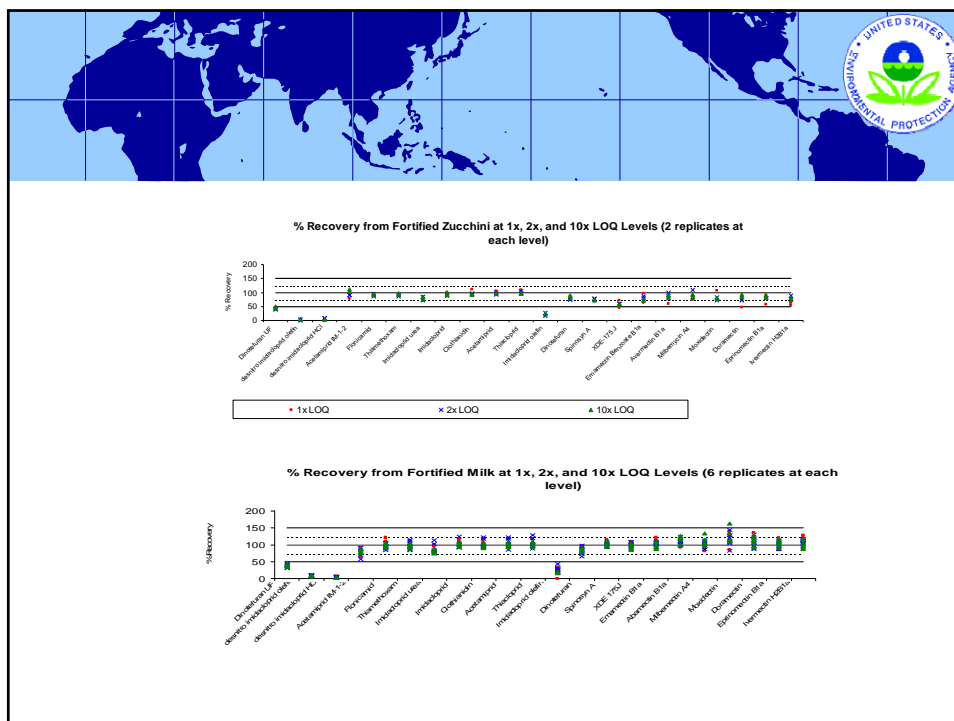


% Recovery from Fortified Strawberry at 1x, 2x, and 10x LOQ Levels (4 replicates at each level)



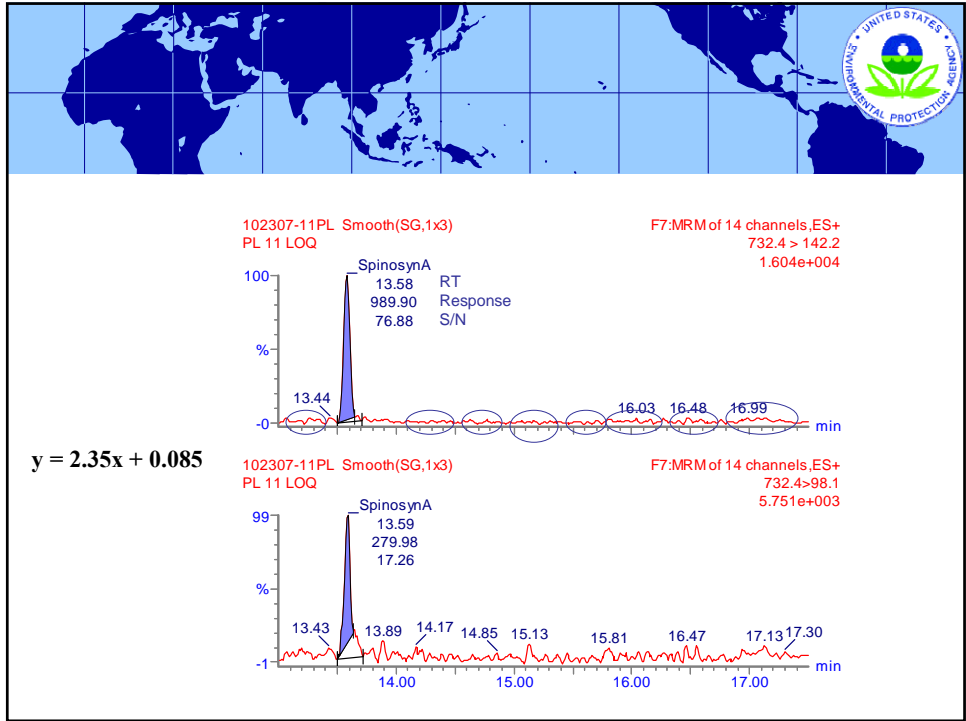
% Recovery from Fortified Watermelon at 1x, 2x, and 10x LOQ Levels (4 replicates at each level)





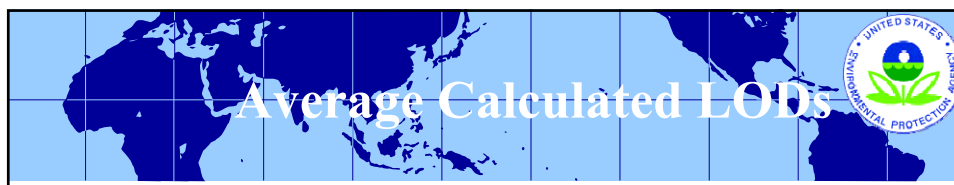
LOD and LOQ: *following Mass Lynx algorithm*

- **Calculated LOD & LOQ:**
 - **Noise:** the standard deviation of regions of the trace that consists of noise; could be manually or automatically calculated.
 - **LOD and LOQ concentrations:** calculated by applying the compound's calibration curve equation $y=mx+b$ to the noise response to obtain a value (x) for the concentration.
 - **LOD concentration is 3 * (x)**
 - **LOQ concentration is 10 * (x)**



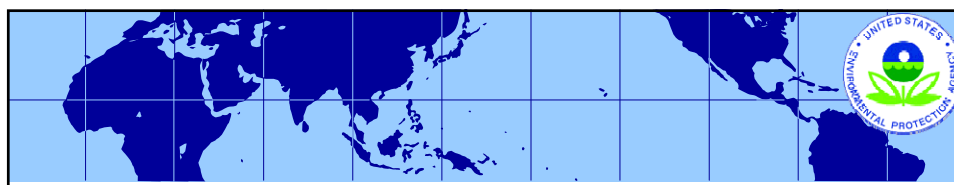
Limits of Detection (LOD) ng/g (ppb)

Macrocyclic lactone	Target LOD	Estimated LOD	Calculated LOD (average)
1. Avermectin B1a	0.01	0.33	0.178
2. Emamectin B1a	0.10	0.016	0.004
3. Spinosyn A	50	0.006	0.002
4. Spinetoram		0.006	0.002
5. Milbemycin A4		0.66	0.917
6. Moxidectin		0.33	0.329
7. Doramectin		0.33	0.302
8. Eprinomectin		0.33	0.263
9. Ivermectin		0.166	0.197



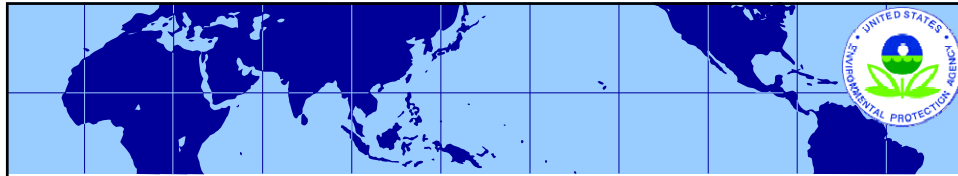
Average Calculated LODs

Macrocytic lactone	Apple	Orange	Spinach	Bean	W-melon	St-berry
	10	8	6	2	4	4
Spinosyn A	0.001	0.003	0.001	0.002	0.002	0.001
Spinetoram	0.002	0.003	0.001	0.002	0.002	0.002
Emamectin Benzoate B1 _a	0.007	0.003	0.002	0.004	0.003	0.002
Avermectin B1 _a	0.120	0.272	0.135	0.148	0.190	0.100
Milbemycin A4	0.639	0.972	0.570	0.823	0.945	0.427
Moxidectin	0.175	0.288	0.262	0.411	0.303	0.107
Doramectin	0.193	0.286	0.175	0.234	0.212	0.114
Eprinomectin B1 _a	0.353	0.312	0.247	0.261	0.276	0.110
Ivermectin H ₂ B1 _a	0.113	0.206	0.104	0.153	0.153	0.094
Macrocytic lactone	Plum	Zucchini	Broccoli	Tomato	Grape	Milk
	2	4	2	4	2	6
Spinosyn A	0.001	0.005	0.001	0.002	0.002	0.002
Spinetoram	0.001	0.002	0.002	0.002	0.005	0.003
Emamectin Benzoate B1 _a	0.005	0.002	0.001	0.003	0.007	0.004
Avermectin B1 _a	0.111	0.139	0.078	0.179	0.302	0.367
Milbemycin A4	0.332	0.437	0.961	0.852	1.992	2.056
Moxidectin	0.135	0.235	0.179	0.270	0.822	0.757
Doramectin	0.128	0.248	0.152	0.300	0.682	0.905
Eprinomectin B1 _a	0.120	0.216	0.107	0.315	0.414	0.420
Ivermectin H ₂ B1 _a	0.086	0.181	0.124	0.236	0.525	0.384



Summary

- **Current CDFA method: not successful in recovering some of the macrocytic lactones.**
- **Developed method: successful in combining two widely different classes of compounds into a single analysis and may be successful in recovering other pesticide classes.**
- **Method: only one modification to the current CDFA method (Add 1% TEA to extraction solvent)**
- **Target LODs: all reached except for Avermectin B1a (Target LOD 0.01 ppb, calculated LOD 0.18 ppb).**
- **Average recovery percentages: mostly between 70-120%, except for the neonicotinoid metabolites and standard deviations were generally <20%.**



Future Steps

- **Adding other analytes to method**
 - Consultation with PDP-CA lab to try the modified method on a matrix spike sample containing 134 pesticides
- **PDP labs might move to QuEChERS**
 - ACL is in the process of checking whether the QuEChERS or its modified version(s) could be applicable to the neonicotinoids and metabolites as well as the macrocyclic lactones