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The Determination of Triclopyr and 2,4-D in Plant Materials

Scope: This method is for the analysis of triclopyr and 2,4-D in acorn, beargrass, huckleberry, maidenhair fern, manzanita berries, willow and yarrow. The reporting limit is 0.05 ppm for all plant material except manzanita berries, which is 0.03 ppm and yarrow, which is 0.1ppm.

Principle: The plant materials were chopped into small pieces and homogenized in a cuisinart with dry ice. Triclopyr and 2,4-D were extracted from the ground sample by blending with benzene and sulfuric acid. An aliquot of the benzene extract was cleaned up by extraction with the sodium bicarbonate solution and ethyl ether. The extract was acidified with sulfuric acid. Methylene chloride was used to extract the residue from the acidified aqueous solution. The resulting extract was concentrated then derivatized with diazomethane and quantitated by GLC/ECD.

Reagents:

1. Combination standard of Triclopyr, CAS# 55335-06-3,and 2,4-D, CAS # 94-75-7, 0.5 mg/mL in acetone obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture).
2. Benzene, pesticide residue grade
3. Ethyl Ether, pesticide residue grade
4 Hexane, pesticide residue grade
5. Methylene chloride, pesticide residue grade
6. Iso-octane, pesticide residue grade
7. Sulfuric acid (1:1), reagent grade
8. Sodium bicarbonate solution, 4% (w/v)
9. Sodium Sulfate, anhydrous, granular (ACS)
10. Diazomethane
11. Dry ice

Safety:

Benzene is recognized as a carcinogen, review MSDS before handling. Diazomethane is also carcinogenic and an explosive reagent, so MSDS should be reviewed before handling. All general laboratory safety rules must be followed.

Equipment:

1. Nitrogen evaporator Organomation Model # 12
2. Rotary evaportor (Büchi/Brinkmann, R110)
3. Cuisinart™ food processor (Model DLC 7)

Equipment: continued

4. Sorvall® Omi-Mixer - pint mason jars
5. Separatory funnel, 500 mL
6. Flat-bottomed round flask, 500 mL
7. Graduated conical centrifuge tubes, 15 mL
8. Mixing cylinder, 100 mL
9. Filter paper, Whatman # 1

Instrument:

Hewlett Packard Gas Chromatograph Model 6890 with autosampler and an electron capture detector (ECD).

Interference:

There are no interferences for triclopyr and 2,4-D in the background material and samples analyzed at this time.

Standard Preparation:

1. The combination 0.5 mg/mL standard was diluted to 100 ug/mL and 10 ug/mL with hexanes for spiking purpose.
2. The combination 0.5 mg/mL standard was derivatized and diluted into a series of desired standard sets that will be used for instrument calibration and sample calculation.

Sample Preservation and Storage:

1. Check the temperature of samples upon arrival and record it.
2. Sign the chain of custody and obtain the EMON number from supervisor.
3. Store all samples waiting for analysis in freezer.

Procedure:

1. Cut entire plant sample into small pieces. Grind the sample in a Cuisinart with dry ice until the sample becomes homogeneous.
2. Transfer the ground sample to a mason jar and cover it with a piece of aluminum foil and apply lid loosely. Store in a freezer overnight to allow carbon dioxide to dissipate.
3. Weigh 20 g of ground acorn, beargrass, huckleberry, maidenhair fern, manzanita berries, willow or yarrow sample into a pint size mason jar. Then add 100 mL of benzene and 1.5 mL of 1:1 sulfuric acid.
4. Blend with Omi-mixer for 4 minutes at a setting of 3.5.
5. Filter the extract through a funnel lined with # 1 Whatman filter paper containing 10 g sodium sulfate into a graduated mixing cylinder.
6. Remove a 50 mL aliquot of extract from the cylinder to a 500 mL separatory funnel.
7. Extract with 200 mL of 4% sodium bicarbonate solution by shaking for 1.5 minute, venting often to relieve pressure. Drain lower aqueous layer into a 600 mL beaker.
8. Add another 100 mL of sodium bicarbonate solution to separatory funnel and shake 1minute. Add lower aqueous layer to the beaker and discard benzene in a proper waste container.
9. Pour contents of beaker back into separatory funnel and add 100 mL ethyl ether. Shake gently for 1 minute and vent often.
10. Drain aqueous layer into the beaker and discard ether.

Procedure: continued

11. Add 3 mL of 1:1 sulfuric acid to aqueous extract carefully and swirl. **Beware-- there will be foaming!** Continue adding sulfuric acid until aqueous solution is acidic (~ 10 mL) and foaming has stopped.
12. Pour acidifed aqueous solution back into separatory funnel.
13. Add 100 mL methylene chloride and shake vigorously for 1 minute.
14. Allow layers to separate. Drain the organic layer into a 500 mL flask.
15. Repeat steps 12 and 13 two more times using 80 mL methylene chloride.
16. Add 5 mL of iso-octane to the flask.
17. Rotoevaporate the extract to ~ 4 mL at 35 °C under approximately 15 inches of Hg vacuum.
18. Add 1 mL diazomethane solution into the flask. Cover the flask with aluminum foil and swirl it gently. Allow the reaction mixture to stand in fumehood for 30 minutes. (If the brownish-yellow color has disappeared within 30 minutes, add additional diazomethane solution and let the reaction mixture stand for another 30 minutes.)
19. Evaporate the solvent and the excess reagent to just dryness at ambient temperature using a gentle stream of nitrogen.
20. Pipet 5 mL of hexane into flask and swirl. Transfer the extract immediately to an autosampler vial for GLC analysis.

Instrument Condition:

Hewlett Packard 5890 GC with ECD
Column: HP-1 (Crosslinked methyl silicone gum) 30 m x 0.53 mm x 0.88 μm
Carrier gas: Helium, column flow rate 1.5 mL/min
Injector temperature: 220 °C
Detector temperature: 300 °C
Column oven temperature:

Ramp 1	Initial temperature:	150 °C hold for 2 min
	Rate:	5 °C / min
Ramp 2	Initial temperature	190 °C
	Rate	30 °C / min
	Final temperature	250 °C hold for 3 min

Injection volume: 1 μL
Retention times: 2,4-D: 7.89 ± 0.10 min
Triclopyr: 9.08 ± 0.10 min

Analysis:

Quality Control:

1. A five-point calibration curve of 0.05, 0.1, 0.25, 0.5 and 1.0 ηg/μL of triclopyr and 2,4-D was obtained at the beginning and the end of each set of samples.
2. Each sample was analyzed two times to insure reliability of the chromatography. If the signal of the sample was greater than that of the highest concentration of the calibration curve, the sample was diluted within the calibration range and reanalyzed.
3. For each set of samples, one matrix blank and two matrix spikes were included, and each set of samples did not contain more than twelve samples.

Method Detection Limit (MDL)

Method Detection Limit refers to the lowest concentration of analyte that a method can detect reliably in either a sample or blank. This was determined by fortifying seven aliquots of background sample matrix at a 0.1ppm level for triclopyr and 2,4-D and processing through the entire method along with a blank. The standard deviation derived from the 7 spiked samples was used to calculate the MDL using the following equation:

$$MDL = t\,S$$

where:

t is the Students' t value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - α = 0.99), which is 3.143. n represents the number of replicates.

S denotes the standard deviation obtained from replicate analyses.

Results of the standard deviation and the MDL are in appendix I.

Reporting Limit (RL):

It refers to the level above which quantitative results may be obtained.

The MDL and RL for each matrix were tabulated as follow:

	Triclopyr		2,4-D	
Matrix	MDL (ppm)	RL (ppm)	MDL (ppm)	RL (ppm)
Acorn	0.03156	0.05	0.0272	0.05
Beargrass	0.00876	0.05	0.0122	0.05
Huckleberry	0.00653	0.05	0.0071	0.05
Maidenhair fern	0.01092	0.05	0.0127	0.05
Manzanita berries	0.0107	0.03	0.0088	0.03
Willow	0.02407	0.05	0.0171	0.05
Yarrow	0.028	0.1	0.019	0.1

Recovery Data:

Method validation was performed, by spiking the background plant materials with three different levels (0.3, 3.0, and 30 ppm) of triclopyr and 2,4-D for five replicates. Results of the method validation are summarized in appendix II.

Calculations:

$$ppm = \frac{\text{(Peak height of sample) x (Std conc) x (Std vol. injected) x (Initial Vol) x (Final Vol of sample)}}{\text{(Peak height of Std) x (Sample vol injected) x (Sample weight (g)) x (aliquot Vol.)}}$$

Acceptance Criteria:

The samples results were calculated by the chemstation using a piecewise curve. The samples were injected two times and the results compared. If the results between the two injections differ less than 10 % either result can be reported. A change greater than 10% with no known reason requires a third injection.

Discussion:

The background yarrow provided by the Department of Pesticide Regulations for the MDL and validation was different than the yarrow provided for quality control samples. The yarrow used for the MDL and validation had an oily substance upon extraction, which might explain for the lower recovery results. Yarrow used for the quality control samples had no oily substance and the results for the spikes were higher than the validation data. The validation data was used to create control charts for both 2,4-D and triclopyr. The control charts should be used as a guideline only at the lower end since the oily substance found in the background material might have hindered the recoveries for 2,4-D and triclopyr. More data is needed to correct the control chart to reflect a better overview of the different background material sampled. Manzanita berries had similar problem with background differing.

References:

1. Hsu, Jean, *The Determination of Triclopyr in Plant Materials*,1997, California Department of Food and Agriculture Laboratory Services, 3292 Meadowview Road, Sacramento, California 95832
2. *Determination of Phenoxies in Vegetation*, Pesticide Residue Laboratory Method, Center for Analytical Chemistry, California Dept. of Food and Agriculture.

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Appendix I

Triclopyr Spike Results (ppm) for MDL Determination

Spike	Acorn	Beargrass	Huckleberry	Maidenhair Fern	Manzanita berries
1	0.0794	0.06583	0.08760	0.0968	0.0935
2	0.0807	0.0664	0.0863	0.0905	0.0921
3	0.0842	0.0597	0.0846	0.0865	0.0952
4	0.0746	0.0609	0.0882	0.0891	0.0881
5	0.0540	0.0637	0.0889	0.0895	0.0852
6	0.0754	0.0591	0.0869	0.0864	0.0929
7	0.0802	0.0629	0.0830	0.0894	0.0911
SD	0.01004	0.002786	0.002078	0.003473	0.0034
MDL	0.031556	0.008756	0.006532	0.010916	0.0107

Spike	Willow	Yarrow
1	0.0923	0.138
2	0.0718	0.123
3	0.0802	0.127
4	0.0825	0.127
5	0.0728	0.145
6	0.0711	0.144
7	0.0818	0.129
SD	0.007658	0.009
MDL	0.02407	0.028

2,4-D Spike Results (ppm) for MDL Determination

Spike	Acorn	Beargrass	Huckleberry	Maidenhair Fern	Manzanita berries
1	0.0769	0.0642	0.0862	0.0932	0.0782
2	0.0784	0.0636	0.0836	0.0853	0.0777
3	0.0798	0.0598	0.0826	0.0825	0.0786
4	0.0749	0.0583	0.0855	0.0860	0.0731
5	0.0545	0.0629	0.0850	0.0812	0.0723
6	0.0726	0.0530	0.0830	0.0819	0.0783
7	0.0768	0.0603	0.0794	0.0859	0.0791
SD	0.008657	0.003882	0.002285	0.00406	0.0028
MDL	0.02721	0.0122	0.007182	0.01276	0.0088

Appendix I continued:
2,4-D Spike Results (ppm) for MDL Determination

Spike	Willow	Yarrow
1	0.0811	0.113
2	0.0679	0.102
3	0.0751	0.107
4	0.0748	0.108
5	0.0678	0.115
6	0.0665	0.117
7	0.0760	0.105
SD	0.00545	0.006
MDL	0.01712	0.019

Appnedix II *Method Validation Results*

Acorn	Triclopyr		2,4-D	
Spike Level (ppm)	**Result** (ppm)	**Recovery** (%)	**Result** (ppm)	**Recovery** (%)
0.3	0.204	68.0	0.202	67.3
	0.233	77.7	0.230	76.7
	0.233	77.7	0.232	77.3
	0.228	76.0	0.225	75.0
	0.227	75.7	0.229	76.3
3.0	2.34	78.0	2.38	79.3
	2.66	88.7	2.66	88.7
	2.44	81.3	2.50	83.3
	2.25	75.0	2.29	76.3
	2.39	79.7	2.41	80.3
30	26.3	87.3	25.8	86.0
	30.7	102	29.5	98.3
	23.9	79.7	23.8	79.3
	24.0	80.0	25.2	84.0
	27.2	90.7	26.8	89.3

Appendix II: continued

Method Validation Results

Beargrass	Triclopyr		2,4-D	
Spike Level (ppm)	**Result** (ppm)	**Recovery** (%)	**Result** (ppm)	**Recovery** (%)
0.3	0.213	71.0	0.193	64.3
	0.214	71.3	0.188	62.7
	0.247	82.3	0.23	76.7
	0.261	87.0	0.261	87.0
	0.254	84.7	0.254	84.7
3.0	2.2	73.3	1.97	65.7
	2.4	80.0	2.11	70.3
	2.25	75.0	2.05	68.3
	2.46	82.0	2.46	82.0
	2.55	85.0	2.55	85.0
30	21.7	72.3	19.1	63.7
	21.7	72.0	18.3	61.0
	24.7	82.3	20.7	69.0
	24.1	80.3	24.1	80.3
	26.3	87.7	26.3	87.7

Huckleberry	Triclopyr		2,4-D	
Spike Level (ppm)	**Result** (ppm)	**Recovery** (%)	**Result** (ppm)	**Recovery** (%)
0.3	0.227	75.7	0.223	74.3
	0.244	81.3	0.238	79.3
	0.255	85.0	0.24	80.0
	0.249	83.0	0.236	78.7
	0.223	74.3	0.213	71.0
3.0	2.64	88.0	2.64	88.0
	2.43	81.0	2.4	80.0
	2.32	77.3	2.23	74.3
	2.28	76.0	2.21	73.7
	2.61	87.0	2.57	85.7
30	25.0	83.3	24.6	82.0
	24.9	83.0	24.0	80.0
	27.0	90.0	25.8	86.0
	27.0	90.0	25.7	85.7
	25.3	84.3	25.1	83.7

Appendix II: continued
Method Validation Results

Maidenhair Fern	Triclopyr		2,4-D	
Spike Level (ppm)	**Result** (ppm)	**Recovery** (%)	**Result** (ppm)	**Recovery** (%)
0.3	0.279	93.0	0.236	78.7
	0.240	80.1	0.194	64.6
	0.252	84.1	0.205	68.4
	0.281	93.8	0.230	76.6
	0.253	84.4	0.211	70.2
3.0	2.75	91.7	2.51	83.8
	2.47	82.2	2.17	72.4
	2.61	87.1	2.35	78.3
	2.26	75.3	2.01	67.0
	2.27	75.6	2.05	68.5
30	28.4	94.6	26.2	87.3
	25.5	84.9	23.8	79.3
	30.0	100	27.6	92.0
	26.7	88.9	24.3	81.0
	24.0	80.0	22.7	75.5

Manzanita berries	Triclopyr		2,4-D	
Spike Level (ppm)	**Result** (ppm)	**Recovery** (%)	**Result** (ppm)	**Recovery** (%)
0.3	0.236	78.7	0.206	68.7
	0.225	75.0	0.172	57.3
	0.225	75.0	0.192	64.0
	0.251	83.7	0.207	69.0
	0.258	86.0	0.226	75.3
3.0	2.60	86.7	2.42	80.7
	2.17	72.3	1.89	63.0
	2.31	77.0	2.07	69
	2.56	85.3	2.30	76.7
	2.63	87.7	2.36	78.7
30	24.5	81.7	23.3	77.7
	22.0	73.3	20.0	66.7
	22.1	73.7	20.2	67.3
	26.3	87.7	24.9	83.0
	27.6	92.0	25.7	85.7

Appendix II: continued
Method Validation Results

Willow	Triclopyr		2,4-D	
Spike Level (ppm)	**Result** (ppm)	**Recovery** (%)	**Result** (ppm)	**Recovery** (%)
0.3	0.278	92.7	0.25	83.3
	0.266	88.6	0.244	81.2
	0.296	98.8	0.287	95.7
	0.261	86.9	0.245	81.8
	0.263	87.7	0.248	82.7
3.0	2.66	88.6	2.48	82.7
	2.55	84.9	2.36	78.8
	2.75	91.7	2.78	92.8
	2.29	76.3	2.29	76.3
	2.27	75.7	2.22	74.0
30	24.1	80.4	23.5	78.2
	23.4	78.0	23.9	79.7
	26.8	89.3	28.4	94.6
	23.9	79.7	22.4	74.8
	23.5	78.4	22.9	76.2

Yarrow	Triclopyr		2,4-D	
Spike Level (ppm)	**Result** (ppm)	**Recovery** (%)	**Result** (ppm)	**Recovery** (%)
0.3	0.209	69.7	0.176	58.7
	0.206	68.7	0.171	57.0
	0.178	59.3	0.145	48.3
	0.164	54.7	0.127	42.3
	0.196	65.3	0.164	54.7
3.0	1.45	48.3	1.22	40.7
	1.83	61.0	1.52	50.7
	1.96	65.3	1.60	53.3
	1.68	56.0	1.48	49.3
	1.84	61.3	1.68	56.0
30	17.7	59.0	15.7	52.3
	17.6	58.7	16.5	55.0
	17.6	58.7	15.8	52.7
	18.8	62.7	16.1	53.7
	14.5	48.3	18.8	62.7