Title: Determination of Phenoxy Herbicides in Surface water using Gas Chromatography/MSD

1. Scope:

    This section method (SM) documents the selected phenoxy herbicide analysis in surface water by all authorized section personnel.

2. Principle:

    The surface water sample is extracted with methyl tertiary butyl ether. The extract is evaporated on a rotary evaporator and diluted to a final volume of 1.0 mL with acetone. The extract is then analyzed by a gas chromatograph equipped with a mass selective detector (MSD).

3. Safety:

    3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.
    3.2 Methylene chloride is a regulated and controlled carcinogenic hazardous substance. It must be stored and handled in accordance with California Code of Regulations, Title 8, Subchapter 7, Group 16, Article 110, Section 5202.

    3.3 All solvents should be handled with care in a ventilated area.

4. Interferences:

    There are matrix interferences that cause quantitative problems. Therefore the calibration standards will be made up in appropriate matrix.

5. Apparatus and Equipment:

    5.1 Rotary evaporator (Büchi/Brinkman or equivalent)
    5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)
    5.3 Vortex-vibrating mixer
    5.4 Stir plate
    5.5 Balance (Mettler PC 4400) or equivalent
    5.6 Gas Chromatograph equipped with a mass selective detector (MSD)

6. Reagents and Supplies

   6.1   Methyl tertiary butyl ether (MTBE), nanograde or equivalent pesticide grade
   6.2   Methylene Chloride, nanograde or equivalent pesticide grade
   6.3   Acetone, nanograde or equivalent pesticide grade
   6.4   Sodium Hydroxide, ACS grade
   6.5   Hydrochloric acid, ACS grade
   6.6   Diazomethane – prepared from Diazald
   6.7   2,4-D   CAS# 94-75-7
   6.8   MCPA   CAS# 94-74-6
   6.9   Dicamba   CAS# 1918-00-9
   6.10   Triclopyr   CAS# 55335-06
   6.11   Conical tube with glass stopper, 15-mL graduated, 0.1 mL subdivision
   6.12   Separatory funnel, 1 L
   6.13   Boiling flask, 250 mL
   6.14   Disposable Pasteur pipettes, and other laboratory ware as needed
   6.15   Recommended analytical column:

          For MSD - 5% phenyl Methylsilicone (HP-5ms or equivalent) fused silica column,
          30 m x 0.25 mm x 0.25 μm film thickness.

7. Standards Preparation:

   7.1 Dilute the 1 mg/mL phenoxy herbicide standards obtained from the CDFA/CAC
        Environmental Analysis Standards Repository with acetone to make up a series of
        mixed working standards (see 10.2).   These standards shall be prepared to cover
        the linear range from 0.02 µg/mL to 1.0 µg/mL for all phenoxy herbicides.

   7.2 The calibration standards are added to matrix blank extracts (9.1.2.1) to correct for
        matrix background interference.

   7.3 Store standards according to manufacturing requirement. Keep all standards in
        designated refrigerator for storage.

   7.4 The expiration date of each mixed working standard is six months from the
        preparation date or same as stock standards, if sooner.

8. Sample Preservation and Storage:

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All water samples and sample extracts shall be stored in the refrigerator (4 ± 3 °C).

9. Test Sample Preparation:

9.1 Sample Preparation

9.1.1 Remove samples from refrigerator and allow samples to come to room temperature before extraction.

9.1.2 Preparation of matrix blank and matrix spike:

The Department of Pesticide Regulation (DPR) provides the background water for matrix blank and spikes.

9.1.2.1 Matrix blank: Weigh out 400 g of background water and follow the test sample extraction procedure.

9.1.2.2 Matrix spike: Weigh out 400 g of background water. Spike a client requested amount of phenoxy herbicides into the background water and let it stand for 1 minute. Follow the test sample extraction procedure.

9.2 Test Sample Extraction

9.2.1 Weight out 400 g of the sample and transfer into a 600mL beaker.

9.2.2 Adjust the pH of the sample to above pH=12 with conc. Sodium hydroxide. Add 100 ± 10 g sodium chloride. Stir the sample and allow 1 hour for the sample to hydrolyze at room temperature converting all forms of the herbicides to the parent acid.

9.2.3 Stir rapidly on a stir plate with 75 ± 5 mL of methylene chloride for 2 minutes.

9.2.4 Transfer contents of beaker to a 1 liter separatory funnel.

9.2.5 After phases have separated, drain lower methylene chloride layer and discard. Return the water layer to the beaker.

9.2.6 Repeat steps 9.2.3 to 9.2.5 two more times using 60 ± 5 mL of methylene chloride each time. Discarding the methylene chloride.

9.2.7   Adjust the pH of the sample to less than pH=2 with conc. Hydrochloric acid.

9.2.8   Stir rapidly on a stir plate with 75 ± 5 mL of MTBE for 2 minutes.

9.2.9   Transfer contents of beaker to a 1 liter separatory funnel.

9.2.10 After phases have separated, drain lower water layer back into beaker. Transfer the organic layer into a 250mL boiling flask

9.2.11 Repeat steps 9.2.8 to 9.2.10 two more times using 60 ± 5 mL of MTBE each time.

9.2.12 Evaporate the sample extract to 2 - 4 mL on a rotary evaporator using a water bath at 40 ± 2 °C and 15 - 20 inch Hg vacuum. Transfer the extract to a calibrated 15 mL graduated test tube.

9.2.13 Rinse flask 3 more times with 2 - 4 mL of MTBE and transfer each rinse to the same test tube. Let the tubes sit for several hours in a refrigerator to allow the remaining water in the extract to settle on the bottom of the tube.

9.2.14 Remove the tubes from the refrigerator and remove remaining water from the tube using a disposable pipette.

9.2.15 Evaporate the extract to a volume slightly less than 1 mL in a water bath at 40 ± 5 °C under a gentle stream of nitrogen.

9.2.16 Add approxametly 1 mL of diazomethane to each sample, spike, blank and standards. Allow to sit for 15 to 20 minutes to methylate the herbicides. The yellow color of the diazomethane should be evident and persist for this period.

9.2.17 Evaporate the extract to approximately about 0.5 mL in a water bath at 35 ± 5 °C under a gentle stream of nitrogen to remove any remaining diazomethane. Then bring to a final volume of 1.0 mL with acetone, mix well and transfer into an auto sampler vial.

9.2.18 Submit extract for GC/MS analysis.

10. Instrument Calibration:

   10.1 The calibration standards are added to a matrix blank extract to correct for matrix background interference.

   10.2 A calibration standard curve consists of minimum of three levels. Standard concentrations of 0.02, 0.10, 0.20, 0.50, and 1.00 µg/µL are recommended. Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r) equal to or greater than 0.995.

11. Analysis:

   11.1 Injection Scheme

   Recommended injection scheme: calibration standards, Solvent, QC samples, Test Samples (maximum of 10-12 samples) and Calibration standards. Injection an old sample or matrix blank before the sequence analysis to condition the instrument is recommended.

   11.2 GC Instrumentation

   11.2.1 Analyze phenoxy herbicides by a gas chromatograph equipped with mass selective detector.

   11.2.2 Recommended instrument (GC/MSD) parameters: Injector 250 °C; detector 250 °C; oven temperature 80 °C (hold 2 min.) to 180 °C @ 20 °C/min. to 280 °C @ 6 °C/min. (hold 6 min.); injection volume 3 µL.

   Ions Selected for SIM Acquisition:
Dicamba	**203**, 205, 234, 236
MCPA	**141,** 143, 214
2,4-D	**234**, 236, 199
Triclopyr	**210**, 212, 269

   (Quantitation ions are in bold)

12. Quality Control:

   12.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample.

   12.2 The matrix blank should be free of target compounds above the MDL.

   12.3 The recoveries of the matrix spike shall be within the control limits.

      12.3.1 When spike recoveries fall outside the control limits, the chemist must investigate the cause. The entire extraction set of samples may be re-analyzed. If the spike recoveries fall within the limit, then the results from the re-analyzed samples shall be reported.

      12.3.2 If the spike recoveries still fall outside the control limits, the client will be notified. The backup samples will be extracted and analyzed.

   12.4 The retention time should be within $\pm$ 2 percent of that of the standard.

   12.5 The sample must be diluted if results fall outside the linear range of the standard curve.

   12.6 Bracketing standard curves should have a percent change less than 20 %.

   12.7 Method Detection Limits (MDL)

      The method detection limit refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 replicate water samples are spiked at 0.05 pp. The standard deviation from the spiked sample recoveries are used to calculate the MDL for each analyte using the following equation:

      $$MDL = tS$$

      Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicate used to determine the MDL, t=3.143.

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12.8 Reporting limit (RL):

The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. Per client agreement, the RL is chosen in a range 1-5 times the MDL except in special cases.
MDL data and the RL are tabulated in Appendix IA and IB.

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of three sample sets. Each set included five levels of fortification (0.02, 0.1, 0.2, 0.5, and 1.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method.

12.9.2 Upper and lower warning and control limits are set at $\pm$ 2 and $\pm$ 3 standard deviations of the average % recovery, respectively

Method validation results and control limits are tabulated in Appendix IIA and IIB.

12.10 Estimated Measurement Uncertainty:
Total uncertainty for this method is 16% at 95% confidence interval.

12.11 Trend Identification
  12.11.1 All matrix spike recoveries for phenoxy herbicides analysis will be put into control charts and monitored for trends. Three trend characteristics will be evaluated at least bi-yearly by the supervisor or designee.
      2 of 3 points above or below 2/3 of the UCL or LCL.
      7 continuous points above or below the center line (CL)
      14 points alternating above and below the CL.
  12.11.2 When results indicate an out of control situation the supervisor or designee will indicate this on the control chart and take appropriate corrective action, which may include monitoring the results more closely to initiating a formal corrective action with root cause investigation.

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13. Calculations:

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The software uses a linear or quadratic curve fit, with all levels weighted equally. Alternatively, at chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

$$\text{ppb} = \frac{\text{(sample peak ht. or area) (std. conc.) (std. vol. injected) (sample final vol., (mL))(1000 }\mu\text{L/mL)}}{\text{(std. peak ht. or area) (sample vol. injected) (sample wt., g)}}$$

14. Reporting Procedure:

14.1 Identification of Analyte

For responses within calibration range, compare the retention time of the peaks with the retention time of standards. For positive results retention times shall not vary from the standards more than 2 percent.

14.2 Sample results are reported out according to the client's analytical laboratory specifications.

15. Discussion and References:

15.1 Sample response and quantitation vary depending on matrix background in the samples. The calibration standards were added to a matrix blank extract to correct for matrix background interference.

16. References:

16.1 *EPA Method 8151A, Chlorinated Herbicides By GC using Methylation Derivatization*. Test methods for Evaluating Solid Waste, 1986

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APPENDIX IA

The determination of Method Detection Limit (MDL) data and Reporting Limit (RL)

Sample ID	Dicamba	MCPA	2,4-D	Triclopyr
MDL #1	0.059	0.049	0.052	0.054
MDL #2	0.057	0.051	0.048	0.049
MDL #3	0.056	0.050	0.049	0.052
MDL #4	0.069	0.061	0.056	0.062
MDL #5	0.065	0.061	0.058	0.064
MDL #6	0.066	0.067	0.061	0.065
MDL #7	0.067	0.060	0.055	0.058
SD	0.005251	0.006952	0.004741	0.006237
SD* 3.143	0.016503	0.021851	0.014901	0.019604
MDL	**0.017**	**0.022**	**0.015**	**0.020**
RL	0.050	0.050	0.050	0.050

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APPENDIX IIA

Sample ID	Spike level	Set 1	% Rec.	Set 2	% Rec.	Set 3	% Rec	Control Limit
Dicamba	0.0200	0.0209	**104.5**	0.0224	**112.0**	0.0175	**87.5**	Mean:100.9 SD 21.9
	0.1000	0.1241	**124.1**	0.1415	**141.5**	0.1277	**127.7**	UCL: 166.6
	0.2000	0.1729	**86.5**	0.1966	**98.3**	0.1905	**95.3**	UWL: 144.7
	0.5000	0.4032	**80.6**	0.4724	**94.5**	0.4191	**83.8**	LWL: 57.1
	1.0000	0.6894	**68.9**	1.3210	**132.1**	0.7731	**77.3**	LCL: 35.2

Sample ID	Spike level							Control Limit
MCPA	0.0200	0.0229	**114.5**	0.0206	**103.0**	0.0189	**94.5**	Mean:98.0 SD 19.9
	0.1000	0.1164	**116.4**	0.1307	**130.7**	0.1223	**122.3**	UCL: 157.6
	0.2000	0.1649	**82.5**	0.1848	**92.4**	0.1913	**95.7**	UWL: 137.7
	0.5000	0.3878	**77.6**	0.4505	**90.1**	0.4091	**81.8**	LWL: 60.2
	1.0000	0.6698	**67.0**	1.2674	**126.7**	0.7586	**75.9**	LCL: 40.4

Sample ID	Spike level							Control Limit
2,4-D	0.0200	0.0232	**116.0**	0.0238	**119.0**	0.0204	**102.0**	Mean:97.2 SD 21.2
	0.1000	0.1087	**108.7**	0.1335	**133.5**	0.1176	**117.6**	UCL: 170.0
	0.2000	0.1601	**80.1**	0.1652	**82.6**	0.1649	**82.5**	UWL: 139.7
	0.5000	0.3448	**69.0**	0.4097	**81.9**	0.3849	**77.0**	LWL: 54.85
	1.0000	0.6906	**69.1**	1.2182	**121.8**	0.8035	**80.4**	LCL: 33.5

Sample ID	Spike level							Control Limit
Triclopyr	0.0200	0.0238	119.0	0.0224	112.0	0.0201	**100.5**	Mean: 99.2 SD 22.1
	0.1000	0.1189	118.9	0.1379	137.9	0.1283	**128.3**	UCL: 165.5
	0.2000	0.1625	81.3	0.1752	87.6	0.1850	**92.5**	UWL: 143.4
	0.5000	0.3882	77.6	0.4298	86.0	0.4071	**81.4**	LWL: 55.0
	1.0000	0.6565	65.7	1.2182	121.8	0.7772	**77.7**	LCL: 32.9

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