California Department of Food and Agriculture
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Analysis of Dacthal, Dacthal Monoacid, and Dacthal Diacid in Well Water using Gas Chromatography/MSD

1. Scope:

   This section method (SM) documents Dacthal (DCPA), Dacthal monoacid (MTP), and Dacthal diacid (TPA) analysis in well water and is followed by all authorized EMON personnel.

2. Principle:

   The SM describes the method for determination of Dacthal and its metabolites (Dacthal monoacid and Dacthal diacid) in well water. A 40 gram (±0.1 g) aliquot of ground water, which is acidified with sulfuric acid (H_2SO_4) is passed through a HLB solid phase extraction (SPE) cartridge (200 mg). The column is rinsed with 0.18 M H_2SO_4 and the analytes are eluted with diethyl ether (Et_2O). The organic extract is concentrated at 40°C with a gentle stream of nitrogen to ~ 1-2 mL. The concentrated extract is treated with diazoethane for ~10 minutes at room temperature (RT). Then the treated extract is evaporated to dryness. The dried extract is reconstituted with 0.1 mL of acetone followed by 0.9 mL of iso-octane. The extract is then analyzed by a gas chromatograph (GC) 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. Methanol (MeOH), acetone, diethyl ether (Et_2O), and iso-octane are flammable and toxic solvents. They should be handled with care in a ventilated area.

   3.3. Sulfuric acid (H_2SO_4) is highly corrosive. Apply H_2SO_4 to the sample in a fume hood.

   3.4. N-Nitroso-N-Ethylurea is toxic and a possible carcinogen. Handle with care.

   3.5. Potassium hydroxide (KOH) solution is highly corrosive. Use the KOH solution in a fume hood.

   3.6. Diazoethane in Et_2O is flammable and toxic (possible cancer causing). Apply diazoethane to the sample in a fume hood.

4. Interferences:

   There were no matrix interferences that caused quantitative problems during method development and validation.

5. Apparatus and Equipment:

   5.1. Visiprep Solid Phase Extraction Vacuum Manifold (Supelco, Cat# 913-0445)

   5.2. Varian Bond Elut Reservoir, 50 mL and adapter to SPE cartridge

   5.3. Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)

   5.4. Vortex mixer

   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. Dacthal (DCPA) CAS# 1861-32-1e

   6.2. Dacthal monoacid (MTP) CAS# 887-54-7

   6.3. Dacthal diacid (TPA) CAS# 2136-79-0

   6.4. Sulfuric acid, ACS grade or equivalent

   6.5. Diazoethane – prepared from N-Nitroso-N-Ethylurea

   6.6. 50% KOH solution (1:1 w/v) in water

   6.7. Methanol, nanograde or equivalent pesticide grade

   6.8. Diethyl ether, HPLC grade or equivalent

   6.9. Acetone, HPLC grade or equivalent

   6.10. Iso-octane, pesticide grade or equivalent

   6.11. Solid Phase Extraction Cartridge [Waters, Oasis HLB, 6 cc (200 mg)]

   6.12. Conical tube, 15-mL graduated

   6.13. Disposable Pasteur pipettes, and other laboratory ware as needed

   6.14. Recommended analytical column: **For MSD** - 5% phenyl Methylsilicone (HP-5MS UI or equivalent) fused silica column, 30 m x 0.25 mm x 0.25 μm film thickness.

7. Preparation of Diazoethane:

   Diazoethane is prepared from N-Nitroso-N-Ethylurea. Initially setup the glassware for the distillation process, which includes an ice water bath. Then prepare 30 mL of 50% KOH solution (1:1 w/v) in water and weigh ~10 grams of N-Nitroso-N-Ethylurea into a 250 mL reaction flask. Add ~100 mL of diethyl ether (Et_2O) and a magnetic stir bar to the reaction flask. Attach the reaction flask to the condenser and place in water bath and then slowly add 30 mL of the KOH solution. Slowly increase the temperature of the water bath to ~50°C. Between 40-50 °C, the Et_2O containing the diazoethane should start to distill over. Collect the diazoethane in a flask that has been placed in an ice bath. **Under no circumstance should all of the ether be distilled.** If the reaction is still producing a bright yellow – orange solution, then carefully add another ~20 - 25 mL of Et_2O to the reaction flask. To halt the reaction, turn off the heat and add ice to the water bath to reduce the temperature. Remove the collection vessel and add ~20 mL aliquots of the diazoethane solution into a 25 mL amber glass containers. Store the solution at -20°C. Typical recovery is ~60 - 80 mL of the diazoethane solution.

8. Standards Preparation:

   8.1. Individual stock standards of 1.0 mg/mL were obtained from the CDFA / CAC Standards Repository and commercial suppliers. CDFA / CAC Standards Repository obtained the neat standards from either the manufactures or from commercial suppliers of standards.

   8.2. The individual standards were diluted to 10 µg/mL with acetone. A combination standard of the three analytes was prepared at 40 µg/mL from the individual stock solutions at 1 mg/mL.

   8.3. The combination standard at 40 µg/mL was serial diluted to produce the standard curve spiking solutions at the following concentrations: 0.8, 0.4, 0.2, 0.08, 0.04, 0.02, and 0.008 µg/mL in acetone.

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

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

9. Sample Preservation and Storage:

   9.1. Check and record sample temperature upon arrival for ~10% of the samples.

   9.2. Store all samples in designated area in the walk-in refrigerator (less than 5 °C).

9.3. Return samples to the refrigerator immediately after subsample is taken for extraction.

9.4. Sample extracts shall be stored in the refrigerator (4 ± 3 °C).

10. Test Sample Preparation:

10.1. Background Preparation

The Department of Pesticide Regulation (DPR) provided the control well water for background to be used in method validation.

10.2. Sample Preparation

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

10.2.2. Preparation of matrix blank and matrix spike:

10.2.2.1. Matrix blank: Weigh out 40 g (±0.1 g) of background water and proceed to step 10.2.3.2. of section 10.2.3.

10.2.2.2. Matrix spike: Weigh out 40 g (±0.1 g) of background water. Fortify 0.05 mL of DCPA + metabolites (client specified concentration, typically 0.05 mL of 0.16 µg/mL QC spiking solution) into the background water and vortex mix for ~10 seconds. Then, proceed to step 10.2.3.2. of section 10.2.3.

10.2.3. Test Sample Extraction

10.2.3.1. Weight out 40 g (±0.1 g) of the sample and transfer into a 50 mL polypropylene tube.

10.2.3.2. For samples which were determined to be >5 ppb, the sample must be re-extracted by diluting the sample with control matrix to a final weight of 40 g (±0.1 g).

10.2.3.3. Set up two 12 channels solid phase extraction vacuum manifold (Supelco).

10.2.3.4. Add 0.2 mL of 18 M H_2SO_4 to each sample and vortex mix for ~30 seconds.

10.2.3.5. Connect a Waters Oasis HLB 6 cc (200 mg) SPE columns to each channel. Turn off the unused channels of the manifold. Pre-condition the SPE columns by passing ~14 mL of MeOH, by gravity followed by ~7 mL of 0.18 M H_2SO_4. Inhibit the flow and then add ~5 mL of 0.18 M H_2SO_4 to the SPE column. Attach a SPE adapter with reservoir (Varian Bond Elute Reservoir, 50 mL) to each cartridge. Pour the blank, matrix spike and study samples into the corresponding SPE cartridge with large reservoir. Adjust the vacuum for each sample, so the sample is eluting at a rate of 5-10 mL per minute. The typical operating pressure is about 15-20 inch Hg. Maintain at least 1 cm aqueous level in the column until all sample has passed through the cartridge.

10.2.3.6. As soon as the sample has passed through the column, remove the SPE adapter with reservoir and add ~7 mL of 0.18 M H_2SO_4 and continue the extraction until all the rinse has passed through the columns. Make sure all the columns are properly labeled before disconnecting them.

10.2.3.7. After all the H_2SO_4 solution has passed through the SPE column, then apply at least 15 inches of vacuum for 5 minutes to allow excess liquid to be removed.

10.2.3.8. Elute the columns with ~7 mL diethyl ether (Et_2O) and collect into a 15 mL graduated conical test tube. Carefully remove the excess aqueous layer from the bottom of the conical test tube.

10.2.3.9. Evaporate the eluant in a water bath at 40±2°C with a gentle stream of nitrogen to a volume of ~1-2 mL.

10.2.3.10. For the matrix blank samples that will be used for the calibration curve, add 0.05 mL of the appropriate calibration solution (0.08 – 8 µg/mL) to the concentrated eluant of a blank sample.

10.2.3.11. Treat the organic eluate with ~0.5 mL of diazoethane, mix and let stand for 10 minutes at RT. Then continue the evaporation process until dryness. Reconstitute the dried extract with 0.1 mL of acetone and briefly vortex mix. Then add 0.9 mL of iso-octane and vortex mix.

10.2.3.12. If necessary, dilute the final extract from samples with iso-octane or extract from a control matrix sample to be within the calibration curve range. However, the maximum dilution with iso-octane is 1:5 to avoid peak distortion. If a greater dilution is needed, the sample must be re-extracted (see section 10.2.3.2) by initially pre-diluting the sample with control matrix.

10.2.3.13. Submit extract for GC/MS analysis.

11. Instrument Calibration:

11.1. The calibration standard curve consists of a minimum of three levels.

11.2. The recommended concentration levels of standards is as follows: 0.01, 0.025, 0.05, 0.1, 0.25, 0.50 and 1 ppb.

11.3. Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r^2) equal to or greater than 0.990.

12. Analysis:

12.1. Injection Scheme

Recommended injection scheme: Calibration standards, carryover blank, Blank, Matrix spike sample, test samples (maximum of 10-12 samples) and calibration standards. Injection of an old sample, old calibration standard or matrix blank before the sequence analysis to condition the GC column is recommended.

12.2. GC Instrumentation

12.1.1. Analyze the extract by a gas chromatograph equipped with mass selective detector.

12.2.2. Recommended instrument (GC/MSD) parameters: Injector 210°C; detector 230°C; oven temperature 80°C (hold 2 min.) to 280°C @ 10°C/min. to 300°C @ 25°C/min. (hold 2 min.); injection volume 1 µL.

Ions Selected for SIM Acquisition:

Dacthal (DCPA)	299, **301**, 330, 332
DCPA monoacid	299, **301**, 344, 346
DCPA diacid	**313**, 315, 358, 360

(Quantitation ions are in **bold**)

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13. Quality Control:

13.1. Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of each analyte that a method can detect reliably in either a sample of blank. To determine the MDL, 7 control well water samples were spiked at 0.1 ppb and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for DPCA and its metabolites 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 (n-1, $1 - \alpha = 0.99$). n represents the number of replicates and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

The results for the standard deviations and MDL are in Appendix 1.

13.2. Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The reporting limits for DCPA and its metabolites are 0.05 ppb. This reporting limit was chosen after taking into account matrix effects and various sample background that could be encountered.

13.3. Method Validation

The method validation consisted of 5 sample sets. Each set included six levels of fortification (0.05, 0.1, 0.2, 0.5, 1 and 5 ppb) and a method blank. All spikes and method blanks were processed through the entire analytical method. Recoveries for DPCA and its metabolites are tabulated in Appendix 2.

13.4. Control Charts and Limits

Control charts were generated using the data from the method validation. The upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the % recovery, respectively, shown in Appendix 2.

13.5. Acceptance Criteria

13.5.2. Each set of samples will have a matrix blank and a spiked matrix sample.

13.5.2. The retention time should be within ± 2 per cent of that of the standards.

13.5.3. The recoveries of the matrix spikes shall be within the control limits.

13.5.4. The sample shall be diluted if results exceed the calibration curve.

13.5.5. The standard curves at the beginning and end of each sample set should not have a percent change greater than 20%. The % change in response is calculated as follows:

% Change in response = absolute value of slope
(STD curve before – STD curve after) / STD curve before) x 100

13.5.6. The R^2 of each calibration curve shall be ≥ 0.990.

13.5.7. When the above criteria meet, the chemist may report the average of the two injections.

14. 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 as follows:

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

15. Reporting Procedure:

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

16. Discussion:

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.

Extracts of control matrix samples must be used for blank and carryover blank samples. A solvent blank cannot be used as the peak shape of the analytes will be effected.

For samples which were determined to have concentrations above 5 ppb for either DPCA monoacid or DPCA diacid must be re-extracted using a smaller aliquot. The validation experiment only produce data indicating a sample containing of the DPCA monoacid or DPCA diacid at ≤5 ppb could be fully derivatized with diazoethane and successfully diluted with iso-octane.

17. References:

Lee, Paul, EMON Method 20.5, DPCA (Dacthal), MTP and TPA in Ground Water by GC/MSD, 1990, California Department of Food and Agriculture, Center for Analytical Chemistry, Environmental Monitoring Section, Sacramento, California 95832.

APPENDIX 1

MDL Experiment - DCPA and Its Metabolites in Ground H2O

Sample Name	DCPA				DCPA, Monoacid				DCPA, Diacid			
	1st Inj	2nd Inj	Mean Found	Mean % Rec	1st Inj	2nd Inj	Mean Found	Mean % Rec	1st Inj	2nd Inj	Mean Found	Mean % Rec
MDL-0.1-1	76.6	68.6	72.6	72.6	105	92.7	98.9	98.9	91.5	83.8	87.7	87.7
MDL-0.1-2	75.6	70.9	73.3	73.3	98.9	93.1	96.0	96.0	85.7	80.6	83.2	83.2
MDL-0.1-3	79.7	71.9	75.8	75.8	97.3	87.3	92.3	92.3	80.7	73.0	76.9	76.9
MDL-0.1-4	79.8	71.9	75.9	75.9	97.5	88.6	93.1	93.1	78.7	70.7	74.7	74.7
MDL-0.1-5	76.2	70.5	73.4	73.4	89.9	83.4	86.7	86.7	58.8	55.0	56.9	56.9
MDL-0.1-6	78.5	77.0	77.8	77.8	102	98.4	100	100	83.4	81.8	82.6	82.6
MDL-0.1-7	75.3	70.0	72.7	72.7	103	96.7	99.9	99.9	78.9	72.8	75.9	75.9
	Mean % Recovery			**74.5**				**95.3**				**76.8**
	SD			**2.00**				**4.94**				**9.96**
	MDL (ppb*1000)			**6.29**				**15.5**				**31.3**
	MDL (ppb)			**0.00629**				**0.0155**				**0.0313**
	RL (ppb*1000)			**50.0**				**50.0**				**50.0**
	RL (ppb)			**0.05**				**0.05**				**0.05**

MDL=3.143*SD

All values for the mean found, first and second injections are reported as 1000*ppb.

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APPENDIX 2

Cumulative Results During MV for DCPA and Its Metabolites in Ground H2O

		MVR1		MVR2		MVR3		MVR4		MVR5			
Analyte	Spike (ppb)	Mean Found	Mean % Recovery	Mean Found	Mean % Recovery	Mean Found	Mean % Recovery	Mean Found	Mean % Recovery	Mean Found	Mean % Recovery		
DCPA	0.05	39.8	79.6	37.5	75.0	36.9	73.8	38.9	77.8	37.8	75.6	**Mean % Rec**	**70.9**
	0.10	69.8	69.8	74.9	74.9	73.0	73.0	71.0	71.0	69.7	69.7	**SD**	**4.52**
	0.20	137	68.5	141	70.5	147	73.5	153	76.5	131	65.5	**UCL**	**84.5**
	0.50	358	71.6	367	73.4	341	68.2	328	65.6	353	70.6	**UWL**	**79.9**
	1.0	617	61.7	721	72.1	689	68.9	756	75.6			**LWL**	**61.9**
	5.0	3304	66.0	3649	73.0	3082	61.6	3323	66.4	3343	66.8	**LCL**	**57.3**
DCPA, Monoacid	0.05	56.8	114	44.5	89.0	48.8	97.6	46.8	93.6	51.8	104	**Mean % Rec**	**94.1**
	0.10	97.6	97.6	91.8	91.8	103	103	90.4	90.4	99.2	99.2	**SD**	**6.93**
	0.20	196	98.0	177	88.5	198	99.0	185	92.5	194	97.0	**UCL**	**114.8**
	0.50	437	87.4	460	92.0	490	98.0	426	85.2	481	96.2	**UWL**	**107.9**
	1.0	834	83.4	900	90.0	970	97.0	931	93.1			**LWL**	**80.2**
	5.0	3826	76.6	4683	93.6	4533	90.6	4672	93.4	4837	96.8	**LCL**	**73.3**
DCPA, Diacid	0.05	40.6	81.2	40.5	81.0	45.8	91.6	38.2	76.4	45.8	91.6	**Mean % Rec**	**76.2**
	0.10	78.7	78.7	70.1	70.1	84.8	84.8	69.6	69.6	82.3	82.3	**SD**	**9.21**
	0.20	169	84.5	140	70.0	173	86.5	144	72.0	175	87.5	**UCL**	**103.8**
	0.50	326	65.2	380	76.0	410	82.0	361	72.2	452	90.4	**UWL**	**94.6**
	1.0	715	71.5	750	75.0	805	80.5	728	72.8			**LWL**	**57.8**
	5.0	3078	61.6	3240	64.8	3146	63.0	3120	62.4	3176	63.6	**LCL**	**48.5**

There may be slight rounding errors due to software transcriptions.

All values for the mean found, first and second injections are reported as 1000*ppb.

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Date	What was revised? Why?