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Dicamba, MCPA, 2,4-D, 2,4,5-T, Triclopyr and Bentazon in River Water by GC/MSD

Scope: This method is for the determination of Dicamba, MCPA 2,4-D, 2,4,5-T, Triclopyr and Bentazon in River water. The reporting limit of this method is 0.1 ppb for all compounds.

Principle: The water sample is acidified below pH 1. The protonated Dicamba, MCPA 2,4-D, 2,4,5-T, Triclopyr and Bentazon are extracted with 1:1 petroleum ether : diethyl ether. The residues are derivatized with diazomethane, and analyzed by gas chromatography on a capillary column using a mass selective detector (MSD).

Reagents and Equipment:

Reagents:

1. Petroleum ether, grade suitable for pesticide residue analysis.
2. Diethyl ether, grade suitable for pesticide residue analysis.
3. Sulfuric acid, concentrated, A.C.S. reagent grade.
4. Hydrochloric acid, concentrated, A.C.S. reagent grade.
5. Ethanol, 95%.
6. Potassium hydroxide, A.C.S reagent grade.
7. N-methyl-1-nitroso-p-toluenesulfonamide, Aldrich D2,800-0
8. Sodium sulfate, anhydrous, suitable for pesticide residue analysis.
9. Diazomethane (see below)
10. Citral, 95% mixture of cis and trans.

Equipment:

1. Rotary evaporator (Büchi/Brinkmann, R110).
2. Nitrogen evaporator (Organomation Model #12).
3. Distillation kit (Aldrich Z 10025-0)
4. Hotplate with magnetic stirrer, 10"x10"
5. Balance, Mettler PC 4400

PREPARATION OF DIAZOMETHANE:

Diazomethane is Explosive and Carcinogenic-use caution and protective measures (read MSDS)

Preparation of Diazomethane: continued

Diazomethane is prepared from N-methyl-1-nitroso-p-toluenesulfonamide. Assemble a (cat #Z10,025-0) distillation apparatus according to the Aldrich Technical Information Bulletin number AL-131.

The reaction flask is placed in a 65°C water bath on a hot plate with a magnetic stirring control. A 0.5-inch stirring bar is placed in the reaction flask and a 1-inch stirring bar is placed in the water bath. Both magnetic bars should be stirring. Place a separatory funnel in the side arm of the Claisen adapter. Add 10 mL of 95% ethanol to a solution of 5 g KOH in 8 mL water in the reaction flask. Five grams of N-methyl-1-nitroso-1-toluenesulfon amide crystals are carefully dissolved in 100 mL ether and transferred into the separatory funnel. The crystals are moderately soluble in ether. Carefully open the stopcock of the funnel to allow the solution to drain into the reaction flask at a slow rate of about 1 hour for the entire 100 mL solution. Add an additional 20 mL of ether to rinse the separatory funnel and drain it into the reaction flask. Diazomethane formed in the reaction is distilled, condensed and collected into a 500 mL flask in an ice bath. After completing the distillation, transfer the diazomethane solution to a 4 ounce brown bottle with a Teflon-lined cap and store it in the freezer. This solution should be good for about a month in the freezer.

Analysis:

Sample Preparation:

1. Wash all glassware with 1N HCl, rinse with deionized water and dry them in a 90°C oven.
2. Allow sample to equilibrate to ambient temperature. Measure 800 mL (or by weight) of the sample to be analyzed into a 1-liter separatory funnel and record the volume or the weight to one decimal point.
3. Add 2.5 mL of the concentrated sulfuric acid to the water slowly and mix well.
4. Add 150 mL of 1:1 petroleum ether : diethyl ether (v/v). Shake it vigorously for 1.5 minutes. ***Vent frequently as pressure builds rapidly.***
5. Allow the phases to separate. Drain the aqueous layer into a 1-liter beaker.
6. Pour the organic phase from the top of the separatory funnel into a 500-mL acid-washed beaker. Transfer the aqueous phase back to the separatory funnel.
7. Repeat steps 4 through 6 twice. Combine the extracts.
8 Add approximately 20 mL of anhydrous sodium sulfate to the solvent extracts and immediately stir with a Teflon rod to remove any water.
9. Pour the dried solvent to an acid-washed 500-mL boiling flask.
10. Rinse the beaker with 20 mL of the 1:1 ether mix and combine in the flask.
11. Evaporate the solvent to about 1-3 mL on a rotary evaporator at 35° C and 20 inches of vacuum.

Derivatization of the Residues:

1. Add 2 mL of the diazomethane solution to the residue in the flask.
2. Allow the reagent to contact the inside surface of the flask by swirling gently and let the reaction mixture sit in fume hood covered with aluminum foil for 20 minutes. (If the brownish-yellow color has disappeared within 20 minutes, add additional diazomethane and let the reaction mixture sit for another 20 minutes.

Derivatization of the Residues: continued

3. Evaporate the solvent and the excess reagent to just dryness at ambient temperature using a gentle stream of nitrogen.
4. Pipette 2 mL ethyl acetate into the flask and swirl. Make sure no significant solvent evaporation occurs before transferring the sample to an autosampler vial. Add 20 µL of 95 % Citral solution into the autosampler vial. The extract is ready for GC analysis.

Instrument Conditions:

Hewlett-Packard Model 6890 Gas Chromatograph equipped with a series 6890 Mass Selective Detector

Column: HP-5MS (5% Phenyl Methyl Siloxane), 30 m X 0.25 mm X 0.25 um film.

Carrier: Helium, 8.8 psi

Column oven temperature:

Initial temperature:	70°C hold for 1.0 minute
Program Rate	15°C/minute
Final	250°C hold for 4 minutes

Injector Temperature: 250°C

Transfer Line Temperature: 280°C

Ions Selected for SIM Acquisition:

Dicamba	188, 203, 234	start time: 6.0 min.
MCPA	141, 214, 216	start time: 9.1 min.
2,4-D	199, 234, 236	start time: 9.7 min.
Triclopyr	210, 212, 271	start time: 10.1 min.
2,4,5-T	209, 233, 268	start time: 10.6 min.
Bentazon	175, 212, 254	start time: 11.4 min.

Retention time:

Dicamba	8.7 min.
MCPA	9.1 min.
2,4-D	9.7 min.
Triclopyr	10.2 min.
2,4,5-T	10.8 min.
Bentazon	11.6 min.

Volume Injected: 2 microliter

Calculation:

$$\text{Analyte (ppb)} = \frac{PA1}{PA2} \times \frac{FV}{W} \times SC \times 1000$$

Where:

PA1 = peak area of analyte from injected sample volume
PA2 = peak area of analyte standard
FV = final volume of sample extract (in mL)
W = sample weight (in grams)
SC = standard concentration (in ng/mL)

Method Performance:

Method Detection Limit(MDL)

Method Detection Limit refers to the lowest concentration of analytes that a method can detect reliably in either a sample or blank. This was determined by fortifying seven aliquots of background water with 0.2 ppb of Dicamba, MCPA, 2,4--D, Triclopyr, 2,4,5-T and Bentazon then 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:

$$\mathbf{MDL} = t\,S$$

where:

t is the Student '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.

COMPOUND	S (standard deviation, ppb)	MDL (ppb)
Dicamba	0.020	0.064
MCPA	0.014	0.045
2,4-D	0.013	0.041
Triclopyr	0.014	0.044
2,4,5-T	0.0196	0.062
Bentazon	0.01	0.031

Reporting Limit(RL)

It refers to the level above which quantitative results may be obtained. In this method the reporting limit is 0.1 ppb for all six compounds.

Recovery Data

The analytical method was validated by preparing 5 sets of spike samples. Each set contained four levels of spikes (0.2, 0.5, 2 and 10 ppb) and a matrix blank. the matrix was background water supplied by Dept. of Pesticide Regulation. All samples were processed through the entire analytical method. Recoveries of these compounds are summarized in the table below.

Method Validation Recovery Data:

Chemical Name	Spike Levels (ppb)	Recovery (%)	$\bar{x}$ (ppb)	Standard Deviation (ppb)	n
Dicamba	0.2	85.8	0172	0.022	5
	0.5	94	0.47	0.012	5
	2.0	106	2.11	0.130	5
	10.0	112	11.18	0.634	5

Recovery Data: continued

Chemical Name	Spike Levels (ppb)	Recovery (%)	$\bar{x}$ (ppb)	Standard Deviation (ppb)	n
MCPA	0.2	104	0.207	0.014	5
	0.5	99.2	0.496	0.018	5
	2.0	105	2.106	0.187	5
	10.0	92.4	9.242	0.912	5
2,4-D	0.2	96.3	0.193	0.010	5
	0.5	90.6	0.453	0.035	5
	2.0	100	2.006	0.204	5
	10.0	82.3	8.234	0.932	5
Triclopyr	0.2	110	0.220	0.018	5
	0.5	111	0.554	0.033	5
	2.0	116	2.326	0.236	5
	10.0	95.6	9.560	0.911	5
2,4,5-T	0.2	99.2	0.198	0.004	5
	0.5	95.1	0.475	0.038	5
	2.0	103	2.05	0.099	5
	10.0	98.3	9.834	0.786	5
Bentazon	0.2	102	0.204	0.016	5
	0.5	94.0	0.470	0.055	5
	2.0	97	1.938	0.106	5
	10.0	95.1	9.512	0.972	5

Discussion:

Our experience indicated that with this method all glassware must be rinsed with acid to ensure a decent recovery.

The diethyl ether should be checked for any interfering peaks before using for extraction. If interfering peaks are present in the diethyl ether distillation is recommended.

Considerable peak sharpening was obtained by adding 20 µl of 95% Citral solution to ~1 mL standard and sample extracts before analysis.

References:

Lee, Paul, *MCPA, DICAMBA and 2,4-D in River Water by GC/MSD, 3-22-93,* Environmental Monitoring Method, California Department of food and Agriculture.

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