INTRODUCTION

Quality Management and Analytical Services, Inc. residue method QMAM94002 is applicable for the quantitative determination of residues of MCPA, MCPA DMAS, and MCPA 2-EHE in water by capillary gas chromatography with mass selective detection. The validated limit of quantitation (LOQ) for water is 1.0 ng/mL as determined by Quality Management and Analytical Services, Inc. This report contains the results of an independent laboratory validation of the residue method.

ANALYTICAL

Sample Numbering, Preparation and Storage

Untreated control water samples were obtained from a freshwater well at Wildlife International Ltd. The well is approximately 40 meters deep and is characterized as moderately-hard water. The well water was passed through a sand filter to remove particles greater than approximately 25 μm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. Prior to use, the water again was filtered to remove microorganisms and particles. Unique sample numbers were utilized to identify and track the control samples.

Preparation of Solutions and Standards

Solutions were prepared by following the directions stated in Section I.1 - I.6 of residue method QMAM94002. Standards were prepared by following the directions stated in Section J.1 - J.6 of residue method QMAM94002.

Fortification of Recovery Samples

Control water was fortified as described in Section L.2 of method QMAM94002 at the 1.0, 10 and 100 times the LOQ for MCPA; at the LOQ for MCPA DMAS and MCPA 2-EHE.

Sample Extraction and Analysis

Once water recovery samples were fortified, they were extracted as described beginning in Section L.3 of method QMAM94002 with no exceptions to the method.

Analytical Instrumentation and Equipment

Instrumentation:	Hewlett-Packard Model 5890A gas chromatograph Hewlett-Packard Model 7673 automatic injector Hewlett-Packard Model 5971A mass selective detector Hewlett-Packard Model G1034B data system software
Column:	J&W Scientific fused silica capillary Durabond-5 liquid phase; 30 m × 0.25 mm i.d., 0.25 μm film thickness
Temperature: Column Injector Interface	80°C for 1.0 min. 80°C to 300°C at 30°C/min. 300°C for 5 min. 250°C 280°C
Carrier Gas: Head Pressure	helium 8 psi
Injection Mode: Purge Delay Splitter Flow Septum Purge	splitless 1.5 min. ~50 mL/min. 3 mL/min.
Injection Volume:	2 μL
Detector: Calibration Program Electron Multiplier	electron impact selected ion monitoring (70 eV) maximum sensitivity autotune 1800 volts (approximately 200 volts above autotune)
Ions Monitored: MCPA ME MCPA 2-EHE Dwell Time:	 *m/z* 214 (quantitation) *m/z* 155 (confirmation) *m/z* 216 (confirmation) *m/z* 312 (quantitation) *m/z* 202 (confirmation) *m/z* 200 (confirmation) 70 msec

Representative Calibration Curves

The coefficients of determination (r^2) of the least squares equation describing the detector response as a function of the standard curve were determined. Typical calibration curves for both MCPA-ME and MCPA 2-EHE are illustrated in Figures 1 and 2, respectively. Typical chromatograms of MCPA-ME and MCPA 2-EHE calibration standards are illustrated in Figures 3 and 4, respectively.

Calculation of Percent Recovery

The standard curve was prepared by plotting the MCPA ME or the MCPA 2-EHE concentration (μg/mL) on the abscissa and the respective detector response (peak area) on the ordinate as shown in Figures 1 and 2. Linear regression analysis was applied to the data to determine the concentration in μg/mL with respect to the detector response as shown below.

For example, using the data from Figure 1:

$$Y = mX + b$$

$$\frac{Y - b}{m} = X$$

$$\text{MCPA Conc. } (\mu g/mL) \text{ in Final Solution} = \frac{(\text{peak area}) - (-15557.41)}{1976548.46}$$

MCPA Conc. (ng/mL) in Sample = MCPA Conc. in Final Solution × 40 × additional dilution factor

The net concentration in each recovery sample was determined by subtracting an apparent MCPA concentration in the control sample (if present) from that of the gross MCPA concentration in the recovery sample.

For example, using the data from Figures 5 and 6:

MCPA Conc. (net ng/mL) = MCPA Conc. (gross ng/mL) - MCPA Conc. (control ng/mL)

MCPA Conc. (net ng/mL) = 1.13 (gross ng/mL) - 0.00 (control ng/mL)

MCPA Conc. = 1.13
(net ng/mL)

The percent recovery was determined by dividing the net concentration of each recovery sample by the theoretical concentration added as shown below:

$$\text{Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

Using the data from Figure 6, the percent recovery of MCPA was calculated as:

$$\text{Recovery} = \frac{1.13 \text{ ng/mL Found}}{1.00 \text{ ng/mL Added}} \times 100\%$$

$$\text{Recovery} = 113\%$$

Statistical Treatment of Data

Average recoveries for each analyte in the matrix were calculated by dividing the sum of the percent recoveries by the total number of fortified samples.

Standard deviations for each analyte in the matrix were also determined. The standard deviation was calculated by summing the squares of the individual deviations from the average recoveries, dividing by the number of degrees of freedom, and extracting the square root of the quotient.

METHOD NUMBER: QMAM94002
DATE: December 01, 1994
SUPERSEDES: None

DETERMINATION OF 4-CHLORO-2-METHYLPHENOXYACETIC ACID DIMETHYLAMINE SALT (MCPA DMAS) AS ITS ACID EQUIVALENT, 4-CHLORO-2-METHYLPHENOXYACETIC ACID (MCPA), AND 4-CHLORO-2-METHYLPHENOXYACETIC ACID 2-ETHYLHEXYL ESTER (MCPA 2-EHE) IN WATER SAMPLES BY GAS CHROMATOGRAPHY WITH MASS SELECTIVE DETECTION

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Quality Management and Analytical Services

for the: MCPA TASK FORCE THREE

Edited by

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DowElanco

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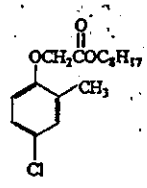
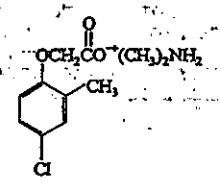
A. Scope

This method is applicable for the quantitative determination of MCPA DMAS, MCPA, and MCPA 2-EHE in water ranging in concentration from a Limit of Quantitation (LOQ) of 1.0 to 1000 ng/mL (Note O.1).

B. Structures



(I)



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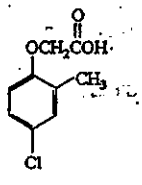
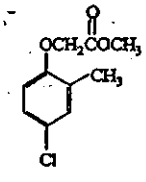


(III)



(IV)

(I) 4-chloro-2-methylphenoxyacetic acid 2-ethylhexyl ester
(II) 4-chloro-2-methylphenoxyacetic acid dimethylamine salt
(III) 4-chloro-2-methylphenoxyacetic acid
(IV) 4-chloro-2-methylphenoxyacetic acid methyl ester (MCPA ME)

C. Principle

A water sample is combined with sodium bicarbonate and extracted twice with hexane. The hexane contains MCPA 2-EHE (FRACTION A) and held for later work-up. The sample is heated to remove residual hexane, acidified, and passed through a pre-conditioned SPE cartridge. The cartridge is dried and MCPA is eluted with methanol/acetone. The eluant is concentrated and derivatized to MCPA ME with BF_3/methanol. The reactants are swamped with water, combined with FRACTION A, and MCPA ME is partitioned into hexane. The hexane is concentrated to a known volume and an aliquot is injected on a GC/MSD for quantitation.

D. Safety Precautions

Each analyst should be acquainted with the potential hazards of the reagents, products, and solvents before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, PRODUCT LITERATURE, AND OTHER DATA. Safety information on products listed in this method should be requested from the supplier.

Disposal of reagents, solvents, and reactants must be in compliance with the laboratory's Standard Operating Procedures (SOPs) and with local, state, and federal laws and regulations.

Exercise normal laboratory precautions when using laboratory reagents which are flammable and/or could be toxic. Flammable solvents must be used away from ignition sources and potentially toxic materials should be used in a hood. Wear appropriate eye, hand, and clothing protection when working with the materials.

Concentrated acids and bases are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

E. Equipment (Note 0.2)

E.1 Balance, Analytical, Model AE 100, 0 to 109 g, Mettler Instrument Corporation, Princeton-Hightstown Road, Hightstown, NJ 08520.

E.2 Balance, Electronic, Top-Loading, Model TP4KD, 0 to 4000 g, O'haus Corporation, P.O. Box 900, 29 Hanover Road, Florham Park, NJ 07932.

E.3 Centrifuge, Model K to accommodate 15-mL centrifuge tubes, International Equipment Company, 300 2nd Avenue, Needham Heights, MA 02194.

E.4 Chemstation, Model B.02.02, Hewlett-Packard Company, 2850 Centerville Road, Wilmington, DE 19808.

E.5 Crimper, catalog number 8710-0979, Hewlett-Packard Company.

E.6 Evaporator, QMAS Model 100, Quality Management and Analytical Services, Inc., Hwy 32 N, Walhalla, ND 58282.

E.7 Gas chromatograph, Model 5890 Series II, equipped with a 5972 mass selective detector, Hewlett-Packard Company.

E.8 Hot plate, 12" x 12", Model 2200, Thermolyne, Curtin Matheson Scientific, Inc., 7677 Equitable Dr., Eden Prairie, MN 55344-3676.

E.9 Shaker, reciprocating, capable of achieving 180 excursions per minute (epm), Model 6000, Eberbach Corporation, 505 S. Maple Road, P.O. Box 1024, Ann Arbor, MI 48103.

E.10 Shaker, vortex, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

E.11 Vacuum manifold, Accu Bond® SPE System, catalog number 280-665, Curtin Matheson Scientific, Inc.

E.12 Water bath, Equatherm, catalog number 273-811, Curtin Matheson Scientific, Inc.

F. Glassware (Note 0.2)

F.1 Bottles, wide-mouth, glass, 200-mL with PTFE-lined caps, I-Chem Research, catalog number 152-926, Curtin Matheson Scientific, Inc.

F.2 Centrifuge tube, conical, glass, 15-mL graduated, Kimax, catalog number 253-822, Curtin Matheson Scientific, Inc.

F.3 Cylinders, graduated, glass, to deliver 100-mL, Pyrex, catalog number 312-404, Curtin Matheson Scientific, Inc.

F.4 Flasks, volumetric, glass, 25-mL, Kimax with ground-glass stopper, catalog number 393-298, Curtin Matheson Scientific, Inc.

F.5 Flasks, volumetric, glass, 100-mL, Kimax with ground-glass stopper, catalog number 104-323, Curtin Matheson Scientific, Inc.

F.6 Flasks, volumetric, glass, 200-mL, Kimax with ground-glass stopper, catalog number 104-331, Curtin Matheson Scientific, Inc.

F.7 Flasks, volumetric, glass, 1000-mL, Kimax with ground-glass stopper, catalog number 104-364, Curtin Matheson Scientific, Inc.

F.8 Pipets, Pasteur-type, disposable, 5 3/4-inch, Kimax, series 72050, catalog number 081-083, Curtin Matheson Scientific, Inc.

F.9 Pipets, graduated, to deliver 10 mL in 1/10-mL increments, Corning, catalog number 250-789, Curtin Matheson Scientific, Inc.

F.10 Pipets, volumetric, Class A, to deliver 1.0 mL, Pyrex, catalog number 250-816, Curtin Matheson Scientific, Inc.

F.11 Pipets, volumetric, Class A, to deliver 4.0 mL, Pyrex, catalog number 250-819, Curtin Matheson Scientific, Inc.

F.12 Pipets, volumetric, Class A, to deliver 5.0 mL, Pyrex, catalog number 250-820, Curtin Matheson Scientific, Inc.

F.13 Pipets, volumetric, Class A, to deliver 10.0 mL, Pyrex, catalog number 250-821, Curtin Matheson Scientific, Inc.

F.14 Pipets, volumetric, Class A, to deliver 15.0 mL, Pyrex, catalog number 250-822, Curtin Matheson Scientific, Inc.

F.15 Pipets, volumetric, Class A, to deliver 20.0 mL, Pyrex, catalog number 250-823, Curtin Matheson Scientific, Inc.

F.16 Pipets, volumetric, Class A, to deliver 25.0 mL, Pyrex, catalog number 250-824, Curtin Matheson Scientific, Inc.

F.17 Pipets, volumetric, Class A, to deliver 50.0 mL, Pyrex, catalog number 190-363, Curtin Matheson Scientific, Inc.

F.18 Stoppers, number 9, ground-glass, Kimax, catalog number 219-113, to fit F.4, Curtin Matheson Scientific, Inc.

F.19 Stoppers, number 13, ground-glass, Kimax, catalog number 219-121, to fit F.5, Curtin Matheson Scientific, Inc.

F.20 Stoppers, number 16, ground-glass, Kimax, catalog number 219-139, to fit F.6, Curtin Matheson Scientific, Inc.

F.21 Stoppers, number 22, ground-glass, Kimax, catalog number 219-154, to fit F.7, Curtin Matheson Scientific, Inc.

F.22 Syringe, glass, 10-µL, Hamilton, catalog number 9301-0725, Hewlett-Packard Company.

F.23 Test tube, glass, 20-mL, threaded, Kimble, catalog number 020-717, Curtin Matheson Scientific, Inc.

F.24 Vials, autoinjector, glass, 2-mL with septa and caps, catalog number 5181-3400, Hewlett-Packard Company.

G. Materials (Note 0.2)

G.1 Adapters, SPE-reservoir, J&W, catalog number 285-302, Curtin Matheson Scientific, Inc.

G.2 Air, compressed, catalog number UN1002, Genex, 700 2nd Avenue, Des Moines, IA 50302.

G.3 Caps, plastic, PTFE lined, size 45-400, catalog number 237-621, to fit F.1, Curtin Matheson Scientific, Inc.

G.4 Caps, phenolic, PTFE lined, size 15-415, catalog number 226-167, to fit F.2 and F.23, Curtin Matheson Scientific, Inc.

G.5 Cartridges, SPE, Accu Bond, catalog number 274-354, Curtin Matheson Scientific, Inc.

G.6 Column, HP-5MS, 0.25 mm x 30 meter, capillary, 0.25 µm film thickness, part number 19091S-433, Hewlett-Packard Company.

G.7 Gloves, clean, lint-free, part number 8650-0030, Hewlett-Packard Company.

G.8 Liner, injection, single-taper, deactivated, part number 5181-3316, Hewlett-Packard Company.

G.9 pH test strips, pH 0-14, ColorpHast, catalog number 393-209, Curtin Matheson Scientific, Inc.

G.10 Reservoir, SPE, 70-mL, J&W, catalog number 700-4008, Curtin Matheson Scientific, Inc.

G.11 Rubber bands, 1/4" x 3-1/2", catalog number 942-7-90064, Quill Corp., P.O. Box 94080, Palatine, IL 60094-4080.

G.12 Sand, sea, E.M. Science, catalog number MSX0076-1, Curtin Matheson Scientific, Inc.

G.13 Septum, 11-mm, low-bleed, catalog number 5181-1263, Hewlett-Packard Company.

G.14 Swabs, cotton, catalog number 259-092, Curtin Matheson Scientific, Inc.

G.15 Vial closures, 11-mm aluminum, PTFE-lined, part number 5181-1210, Hewlett-Packard Company, to fit F.24.

G.16 Weighing paper, 3" x 3", Labcraft, catalog number 340-919, Curtin Matheson Scientific, Inc.

H. Chemicals (Note 0.2)

H.1 Acetone, Omnisolv, Pesticide Residue Quality, E.M. Science, catalog number MAX0116-1, Curtin Matheson Scientific, Inc.

H.2 Ammonium hydroxide, 28 %, E.M. Science, catalog number MAX1303-3, Curtin Matheson Scientific, Inc.

H.3 Boron trifluoride/methanol, 12%, catalog number 26,412-1, Aldrich Chemical Company, 100 West Saint Paul Avenue, Milwaukee, WI 53233.

H.4 Carrier gas, helium, Ultra High Purity, Gerex.

H.5 Hexane, OmniSolv, E.M. Science, catalog number MHX0298-1, Curtin Matheson Scientific, Inc.

H.6 Methanol, OmniSolv, Pesticide Residue Quality, E.M. Science, catalog number MMX0484-1, Curtin Matheson Scientific, Inc.

H.7 PFTBA, 99.9%, for tuning mass spectrometer, catalog number 8500-0656, Hewlett-Packard Company.

H.8 Phosphoric acid, 85%, ACS, Chempure, catalog number 832-536, Curtin Matheson Scientific, Inc.

H.9 Sodium bicarbonate, powder, E.M. Science, catalog number MSX0325-5, Curtin Matheson Scientific, Inc.

H.10 Standards, analytical: (Note O.3)

H.10.1 4-chloro-2-methylphenoxyacetic acid 2-ethylhexyl ester
H.10.2 4-chloro-2-methylphenoxyacetic acid dimethylamine salt
H.10.3 4-chloro-2-methylphenoxyacetic acid
H.10.4 4-chloro-2-methylphenoxyacetic acid methyl ester

H.11 Water, deionized (DI), Culligan, reverse osmosis, activated charcoal filter, and deionizer resin tanks, Culligan Water Conditioning, 416 Gateway Drive, Grand Forks, ND 58102.

I. Reagents (Note O.2)

I.1 Ammonium hydroxide/methanol solution, 5 %: Add 10 mL of 28 % ammonium hydroxide solution to a 200-mL volumetric flask. Dilute to volume with methanol and mix.

I.2 Methenol, acidic, 20 %: Add 50 mL of methanol to a 100-mL volumetric flask, add 20.0 mL of 85 % phosphoric acid and mix. Dilute to volume with methanol and mix.

I.3 Acetone/hexane solution, 1 %: Add 10.0 mL of acetone to a 1000-mL volumetric flask. Dilute to volume hexane and mix.

I.4 Methanol/acetone solution, 10 %: Add 20.0 mL of methanol to a 200-mL volumetric flask. Dilute to volume with acetone and mix.

I.5 Phosphoric acid solution, 1.5 %: Add 500 mL of DI water to a 1000-mL volumetric flask, add 15.0 mL of 85 % phosphoric acid and mix. Dilute to volume with DI water and mix.

I.6 Sodium bicarbonate solution, 1.0 N: Weigh 84.0 g of sodium bicarbonate into a 1000-mL volumetric flask, add 500 mL of DI water and mix until dissolved. Dilute to volume with DI water and mix.

J. Preparation of Standards

Fortification standards:

J.1 MCPA 2-EHE analytical standard. Weigh out 0.1000 g MCPA 2-EHE analytical standard onto a piece of weighing paper and transfer to a 100-mL volumetric flask. Rinse the weighing paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000 µg/mL stock solution.

J.2 MCPA DMAS analytical standard. Weigh out 0.1224 g MCPA DMAS analytical standard onto a piece of weighing paper and transfer to a 100-mL volumetric flask. Rinse the weighing paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1224 µg/mL stock solution equivalent to 1000 µg/mL MCPA (Note O.4).

J.3 MCPA analytical standard. Weigh out 0.1000 g MCPA analytical standard onto a piece of weighing paper and transfer to a 100-mL volumetric flask. Rinse the weighing paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1000 µg/mL MCPA stock solution.

J.4 Take the 1000 µg/mL solutions from J.1 to J.3 and serially dilute in methanol as given in table J.4. Each standard can be prepared separately or in combination by combining 1 mL of each 1000 µg/mL solution from J.1, and either J.2 or J.3 in a 100-mL volumetric flask and bringing it to volume with methanol (Note O.5).

Fortification Solutions:

(1) Conc. of Initial Solution µg/mL	(2) Aliquot of Initial Solution mL	(3) Final Volume of Diluted Solution mL	(4) Conc. of Final Solution µg/mL	(5) Fortified Conc. in Sample (a) ng/mL
1000	10	100	100	1000
100	10	100	10	100
10.0	10	100	1.0	10.0
1.0	10	100	0.10	1.0

a) 1 mL of the solution in column 4 per 100 mL of water sample equals the concentration given in column 5.

J.5 MCPA methyl ester analytical standard. Weigh out 0.1069 g MCPA ME analytical standard onto a piece of weighing paper and transfer to a 100-mL volumetric flask. Rinse the weighing paper with methanol and transfer the rinsate to the volumetric flask. Dilute to volume with methanol to prepare a 1069 µg/mL stock solution equivalent to 1000 µg/mL MCPA (Note O.6).

J.6 Dilute the 1000 µg/mL stock solution of MCPA ME and MCPA 2-EHE with hexane in the following manner to obtain a series of calibration standards that contain both analytes from one-half the LOQ to 10 times the LOQ (Note O.7).

Conc. of Initial Solution µg/mL	Aliquot of Initial Solution mL	Final Volume of Diluted Solution mL	Conc. of Final Solution (a) µg/mL
1000	1.0	100	10
10.0	25.0	100	2.5
2.5	10.0	100	0.25 (b)
10.0	1.0	100	0.10 (b)
0.25	20.0	100	0.050 (b)
0.25	10.0	100	0.025 (b)
0.25	5.0	100	0.0125 (b)(c)

(a) the MCPA ME standard concentrations are equivalent to MCPA
(b) these are the series of standards that make up the calibration curve.
(c) this standard is equivalent to one-half the LOQ and is also included in the series of standards that make up the calibration curve.

K. Instrument Operating Conditions

K.1 Set up the instrument using manufacturers specifications. Inlet liner, septum, and column should be installed on the split/splitless port of the GC/MSD according to manufacturers specifications using lint-free gloves.

K.2 Perform an autotune on the instrument before the analysis of a set of samples. The ions at m/z 69, 219, and 502 from perfluorotributylamine (PFTBA) are used to autotune the instrument. The autotune adjusts MS parameters and calibrates the mass axis so that the instrument will achieve maximum performance. Results from the autotune report should be compared on a daily basis to point out drifts or the need for ion-source cleaning.

K.3 The analysis of the target analytes is performed in the selected-ion-monitoring (SIM) mode. The ions to be monitored for MCPA ME and MCPA 2-EHE are shown below:

Analyte	Quantitation Ion	Qualifier Ion 1	Qualifier Ion 2
MCPA ME	214	155	216
MCPA 2-EHE	312	202	200

K.4 Typical (GC/MSD) operating conditions for the analysis of MCPA ME and MCPA 2-EHE are summarized below:

Instrumentation	Hewlett-Packard Model 5890 Series II Gas Chromatograph/Model 5972 Mass Selective Detector
Column	HP-5MS, 0.25 mm i.d. x 30 m, 0.25-µm film thickness
Oven Temperature	Hold at 80 °C for 1 min, then ramp from 80 °C to 300 °C at 30 °C/min, then hold 5 min.
Injector Temperature	250 °C
Transfer Line Temperature	280 °C
Carrier Gas	Helium
Carrier Gas Flow Rate	1 mL/min
Head Pressure at 50°C	8 psi
Injection Mode	Splitless
Injection Liner	Silanized single-taper
Injector Purge Delay	1.5 min

Septum Purge	100 mL/min (Helium)
Injection Volume	2 µL
Ionization Potential	70 eV
Electron Multiplier Voltage	1400 to 1900 V (typical)
Dwell Time	80 msec

K.5 Mass spectra for MCPA ME and MCPA 2-EHE are shown in Figures 1 and 2, respectively.

K.6 Confirmation

K.6.1 Inject the series of calibration standards described in Section *I.6* and determine the peak area/height for the quantitation and qualifier ion for each analyte, e.g. MCPA ME (m/z 214, 155).

K.6.2 For each standard of each analyte (Section K.3), calculate the confirmation ratio. The average confirmation ratio of the standards will be used to confirm the presence of each analyte in the water samples.

for MCPA ME:

$$\textit{Confirmation Ratio} = \frac{\textit{Peak Area of Confirmation Ion}}{\textit{Peak Area of Quantitation Ion}}$$

$$\textit{Confirmation Ratio} = \frac{\textit{Peak Area/Height at m/z 155}}{\textit{Peak Area/Height at m/z 214}}$$

i.e. $$\textit{Confirmation Ratio} = \frac{2028}{2563}$$

$$\textit{Confirmation Ratio} = 0.79$$

The presence of each analyte is confirmed when the confirmation ratio for the analyte in the sample is within ± 20 % of the average found for the standards.

Any of the three ions listed in K.3 for each analyte can be used as the quantitation or confirmation ion in the event that interference is observed in the quantitation or qualifier ions.

L. Recovery of MCPA DMAS, MCPA, and MCPA 2-EHE from Water

L.1 Place 100-mL of sample into a series of 200-mL wide mouth bottles.

L.2 Retain one sample as a control and fortify the remaining samples with the appropriate aliquot of standard solution as shown in the table in J.4.

Treat each sample as follows:

L.3 Add 10 mL hexane.

L.4 Add 5 mL 1 N $NaHCO_3$ and cap.

L.5 Shake samples on a reciprocating shaker at 180 epm for 10 min.

L.6 Transfer the hexane layer to a 15-mL conical tube.

L.7 Repeat steps L.3 and L.5.

L.8 Transfer the hexane layer and combine with the hexane in L.6.

L.9 Using the QMAS evaporator, concentrate the hexane to 4.0 mL at 50 °C under 150 mL/min air flow (Note O.8) and label FRACTION A.

This solution contains the MCPA 2-EHE and will be combined with the MCPA ME for final quantitation.

L.10 Place the water from L.8 on a sand bath at 80 °C for one hour to remove residual hexane.

L.11 Add 1 mL of 85 % phosphoric acid (Note O.9), cap, and mix vigorously by hand for 30 seconds (Note O.10).

L.12 Prepare a Accu Bond® C18 SPE column by placing a 70-mL reservoir on top of a 6-mL 1000-mg SPE cartridge using an adapter, set the vacuum at 5 inches Hg, and condition the column at approximately 1 drop/sec with 10 mL of methanol followed by 10 mL of 1.5% H_3PO_4. Do not let the column go dry.

L.13 Quantitatively transfer the sample from L.11 to the 70-mL reservoir using two 5-mL aliquots of 1.5 % H_3PO_4 solution to rinse the bottle. Pass the sample through the column at 2 drop/sec (4 to 5 mL/min). After the sample has passed through the column, remove the reservoir and adapter, and remove any water droplets adhering to the SPE column (Note O.11).

L.14 Increase the vacuum to 20 inches Hg and allow the column to dry for 20 min.

L.15 Set the vacuum at 5 inches Hg, add 10 mL of 1 % acetone in hexane and elute at 1 drop/sec until it reaches the top of the C18 packing and discard. Place a 20-mL test tube under the column and elute the MCPA with 5 mL of 10 % methanol in acetone. Discard the C18 column.

L.16 Add 1 mL of 5 % ammonium hydroxide in methanol to the sample in the 20-mL test tube.

L.17 Evaporate the eluate to incipient dryness at 50 °C under 200 mL/min air flow (Note O.8).

L.18 Add 0.5 mL of methanol to azeotrope off the water carried over from the C18 column (Note O.12).

L.19 Add 0.5 mL of 20 % phosphoric acid in methanol.

L.20 Add 1 mL of 12 % boron trifluoride/methanol, cap tightly, and mix by hand for 15 seconds.

L.21 Incubate the sample tube in a water bath at 70 °C for 30 minutes, checking the caps occasionally during the incubation for tightness (Note O.13).

L.22 Remove the tube from the water bath, and cool.

L.23 Quantitatively transfer the reactants from L.22 to FRACTION A using 5 mL of deionized water.

L.24 Cap and shake on a vortex shaker for 5 min. (Note O.14).

L.25 Centrifuge for 3 minutes at 2000 rpm.

L.26 Using a disposable pipet, remove the water layer and discard.

L.27 Mix the sample on a vortex mixer for 2 minutes at high speed (Note O.14).

L.28 Place a portion of the hexane in a 2-mL injection vial, cap, and crimp the vial.

L.29 Inject a 2-µL aliquot on the gas chromatograph for quantitation using a mass selective detector.

L.30 Determine the concentration of the final solution in µg/mL from the standard curve. Typical standard curves for MCPA ME and MCPA 2-EHE are shown in Figures 3 and 4, respectively.

L.31 Calculate the ng/mL of sample by multiplying 40 times µg/mL found in the final solution times any additional dilution factor. The entire formula is provided below:

ng/mL in sample = ng/mL in final solution x 40 x additional dilution factor

$$\textit{Percent Recovery} = \frac{\textit{total ng/mL found (fortified sample)} - \textit{total ng/mL found (control)} \times 100}{\textit{total ng/mL added}}$$

Typical recoveries for MCPA and MCPA 2-EHE can be found in Table I and II, respectively.

M. Determination of MCPA 2-EHE, MCPA DMAS, and MCPA in water

M.1 Begin the analysis with Step L.1 then continue with L.3 through L.29.

M.2 Determine the concentration of the final solution in µg/mL from the standard curve.

M.3 Calculate the ng/mL in the original sample by multiplying 0.40 times µg/mL found in the final solution times any additional dilution factor. The entire formula is provided below:

ng/mL in sample = µg/mL in final solution x 40 x additional dilution factor

M.4 Typical chromatograms of standard, control, and recovery are shown in Figures 5 through 7.

N. Miscellaneous

N.1 A suggested analytical set is as follows (Note O.15):

lowest standard (0.0125 µg/mL)
one reagent blank
one control
one recovery at the LOQ
0.025 µg/mL standard (LOQ)
one recovery at the LOQ
field sample
field sample
0.050 µg/mL standard
field sample
field sample
field sample
0.025 µg/mL standard
field sample
field sample
field sample
field sample
0.10 µg/mL standard
field sample
field sample
field sample
0.25 µg/mL standard
0.025 µg/mL standard

N.2 A typical analytical set consists of sixteen analyses made up of any combination of reagent blank(s), controls, fortified controls, and field samples. These sixteen analytical samples can be completed to encapsulation in one eight-hour day.

N.3 Two convenient stopping points relative to volatilization and degradation are Step L.16 just before evaporation and Step L.29 after encapsulation. In all cases the samples should be refrigerated when stored longer than 12 hours.

O. Notes

O.1 When MCPA DMAS is placed in water it is quantitatively dissociated to MCPA.

O.2 Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests.

O.3 Obtain from Sampling Coordinator, Formulations, DowElanco, P.O. Box 63689, Indianapolis, Indiana 46268-1053.

O.4 The molecular weight of MCPA DMAS is 245.57. The molecular weight of MCPA is 200.63. The ratio of MCPA DMAS to MCPA is 1.224. When 0.1224 g of MCPA DMAS is weighed out it is equivalent to 0.1000 g of MCPA. Weighing out the standards in this manner saves having to make a molecular weight correction for every analytical sample.

O.5 Standards from J.2 and J.3 each contribute MCPA. To avoid duplication of MCPA, only one should be used.

O.6 The molecular weight of MCPA ME is 214.55. The molecular weight of MCPA is 200.63. The ratio of MCPA ME to MCPA is 1.069. When 0.1069 g of MCPA ME is weighed out it is equivalent to 0.1000 g of MCPA. Weighing out the standards in this manner saves having to make a molecular weight correction for every analytical sample.

O.7 The initial dilution of the methanolic solution should not be greater than 1 mL methanol diluted to 100 mL with hexane to overcome any solvent immiscibility problems.

O.8 The evaporation apparatus must be set up in the same fashion each time with conditions carefully controlled.

O.9 Add acid to water carefully.

O.10 The aqueous layer must have a pH less than 2. Check using pH paper. Use more acid if necessary.

O.11 Remove water droplets by using a cotton swab to absorb the droplets. Residual water can inhibit the derivatization process.

O.12 In some cases a small amount of water may remain from the C18 cartridge. This step should be conducted whether or not water remains from step L.18.

O.13 Immerse the tube into the water to the same depth as the liquid in the vial.

O.14 The samples are shaken horizontally by attaching the vials to the platform with rubber bands.

O.15 A standard should be injected at the beginning and end of each sample run and at least every four samples throughout the run. One of the standards must be injected twice during the run, but separated by reagent blanks, controls, or field samples (Refer to N.1).