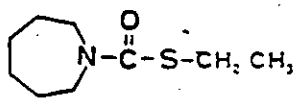
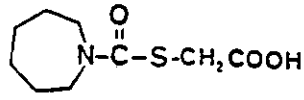
Determination of Carboxymolinate Residues in Water by Gas Chromatography

I. SUMMARY/INTRODUCTION

This method is intended for determining residues in water of carboxymolinate at levels of 0.01 to 3.0 ppm. Carboxymolinate is an aqueous metabolite of molinate, the active herbicidal ingredient in the various formulated products marketed by ICI Americas Inc. under the trademarks "ORDRAM" and "ARROSOLO". The chemical name assigned to molinate by Chemical Abstracts Service is S-ethyl hexahydro-1H-azepine-1-carbothioate [2212-67-1]. The chemical structures are given below.



Molinate



Carboxymolinate

Carboxymolinate is extracted from acidified water samples by shaking with dichloromethane (CH_2Cl_2). The CH_2Cl_2 extract is evaporated to dryness and the remaining residue is treated with trifluoroacetic anhydride and trifluoroethanol to form the trifluoroethyl ester of carboxymolinate. The derivative product is dissolved in toluene and washed with an aqueous solution of sodium bicarbonate. The toluene solution is dried over sodium sulfate and subsequently analyzed by capillary gas chromatography with mass-selective detection.

II. MATERIALS/METHODS

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment with equivalent performance specifications and reagents of comparable purity can be used.

A. Apparatus

1. Gas Chromatograph. Hewlett-Packard model 5880A designed for use with capillary columns and temperature programming of the column oven. The gas chromatograph is equipped with a Hewlett-Packard model 7673 automatic sampler/injector.

2. Mass-Spectrometric Detector. Hewlett-Packard model 5970 mass-selective detector with version 3.2 software.

3. Gas-Chromatographic Column. 10 m by 0.18 mm I.D. capillary column with a 0.3-um film thickness of crosslinked methyl silicone (50% phenyl) and with a minimum of 4800 plates per meter (J&W Scientific, DB-17, catalog no. 121-1713).

4. Vortex Mixer. (VWR, catalog no. K-550-G).

5. Centrifuge. Equipped to accept 50-mL centrifuge tubes (IEC Centra-7; Damon/International Equipment Company, Needham Heights, MA).

6. Centrifuge Tubes. Graduated, 50-mL capacity tubes with no. 16 ground-glass stoppers (catalog no. K-410550; Kontes, Vineland, NJ).

7. Evaporator. Equipped to remove solvents contained in tubes with no. 16 ground-glass openings (Evapo-Mix; Buchler Instruments, Fort Lee, NJ).

8. Syringes. 10-μL capacity (Hamilton 701N) for autosampler and 50-μL capacity (Hamilton 1705N) for fortifications.

9. Dry-Block Heater with Aluminum Heating Blocks. Capable of maintaining 100°C $\pm$ 1°C (Silli-Therm Heating Module with 6 model K Reacti-Blocks, each drilled to fit 5 centrifuge tubes; Pierce Chemical Company, Rockford, IL).

10. Repipet Dispensing Bottles. Rated accuracy 1%, reproducibility 0.1%; for toluene and sodium bicarbonate reagents (Brinkmann Dispensette or Lab Industries Repipet, Fisher).

B. Reagents

1. Solvents. Dichloromethane, toluene, acetone, acetonitrile, and water. All solvents must be of high purity and suitable for use in trace organic analyses by gas chromatography.

2. Carboxymolinate. Analytical reference-standard. Available from ICI Americas Inc., 1200 South 47th Street, Box Number 4023, Richmond, CA 94804-0023; Attention: Environmental Science Department Manager.

3. Trifluoroacetic anhydride, 99+% assay. Aldrich Catalog No. 10,623-2.

4. Trifluoroethanol, 99.5+% assay. NMR grade. Aldrich Catalog No. 32,674-7.

5. Hydrochloric Acid, (conc, 37% wt.). ACS reagent grade.

6. Common Inorganic Salts. Sodium chloride; sodium bicarbonate. ACS reagent grade.

7. Calibration and Fortification Solutions.

To prepare a 1.00 mg/mL (= 1000 µg/mL) stock solution of carboxymolinate, place a known quantity (± 0.1 mg) of approximately 50 mg of primary standard of known purity into a 4-oz narrow-mouth bottle. Calculate the weight of solvent to add, based on the weight of primary standard taken, the purity of the primary standard, and the density of the solvent, as follows:

$$S = \frac{(W \times P \times D)}{L}$$

where S = weight of solvent to add (g),
W = weight of primary standard taken (mg),
P = purity of the standard (100% = 1.00),
D = density of the solvent (g/mL), and
L = required analyte concentration (mg/mL).

Add the calculated weight of the appropriate solvent to the bottle, close the bottle with a "poly-seal" or PTFE-lined cap, and mix thoroughly to dissolve the reference standard. Use acetonitrile (D = 0.782 g/mL at 20°C) for calibration solutions and acetone (D = 0.790 g/mL at 20°C) for fortification solutions.

To prepare working calibration solutions, serially dilute the stock calibration solution by weight with acetonitrile to give 100 µg/mL and 10.0 µg/mL solutions or other concentrations as required. Dilute the stock fortification solution by weight with acetone to give 100 µg/mL and 10.0 µg/mL solutions, or other concentrations as required.

C. Analytical Procedure

1. Extraction

Place a 25-mL subsample of a thoroughly-mixed water sample into a 50-mL centrifuge tube. Add 2 drops of concentrated hydrochloric acid and 5 g of sodium chloride. Vortex mix the contents of the tube to dissolve the NaCl.

Add 5 mL dichloromethane (CH_2Cl_2). Vortex 30 seconds. Centrifuge at approx. 1200 rpm for 1 minute. Transfer the organic (lower) phase to a clean 50-mL centrifuge tube. Repeat CH_2Cl_2 partition and combine organic fractions.

Note: by pre-wetting pipet with CH_2Cl_2 before removing organic layer, carry-over of water is minimized.

2. Fortification

Analyze unfortified and fortified control samples with each sample set to demonstrate method recovery. For example, aliquot 25 mL of untreated control water into a 50-mL centrifuge tube. Add 0.0250 mL of the 10, 100, or 1000 µg/mL fortification solution to produce a fortification level of 0.01, 0.10, or 1.00 ppm. Vortex mix, then add 2 drops conc. HCl and 5 g NaCl. Vortex mix to dissolve NaCl, then proceed with extraction as detailed in section C.1 above.

3. Derivatization

Evaporate CH_2Cl_2 (from Section C.1, above) to dryness using vacuum. Add 0.50 mL trifluoroacetic anhydride and 0.25 mL trifluoroethanol. Cap tightly with a (#16) plastic stopper; vortex mix. Reflux 10 minutes in a 100°C heating block.

Cool to room temperature. Add 4 mL toluene; vortex mix. Add 10 mL 1% sodium bicarbonate solution; vortex mix 1 minute. Centrifuge at approx. 1200 rpm for 1 minute.

Transfer toluene to a 2-dram glass vial containing approx. 1 g anhydrous sodium sulfate. Vortex mix. Transfer a portion of the dried toluene extract to a

GC autosampler vial. Continue with GC/MSD analysis, described below. Seal 2-dram vials with "poly-seal" screw caps and refrigerate toluene extracts when not in use.

D. Instrumentation

1. Operating Conditions

Follow the manufacturer's instructions for operation of the gas chromatograph and mass-selective detector. The specific conditions listed below were used to generate the data and chromatograms presented in this report.

Gas Chromatograph:

Carrier gas:	Helium, oxygen scrubbed.
Column head-pressure:	40 kPa
Inlet type:	splitless; 4 mm i.d. *
Inlet purge flow:	50 mL/min helium
Injection port temp.:	220°C
Interface temp.:	230°C
Initial oven temp.:	70°C
Initial time:	0.5 min
Oven program-rate:	20°C/min
Final oven temp.:	260°C
Volume injected:	2.0 µL
Purge function activated:	0.5 min
Total run time:	11.0 min

* Note for inlet: silanized glass tube lightly packed with silanized glass wool.

Mass-Selective Detector:

Software version:	3.2
Mode:	low resolution s.i.m.
Dwell time:	150 msec
Auto-Tuning:	optics optimized for m/z 69, 219, and 502 with perfluorotributylamine (PFTBA)
Quantitation:	Peak height; external standard
Mass monitored:	m/z 299
Solvent delay:	6.7 min
Detector on:	6.7-8.7 min
EMT Voltage:	2000 V. absolute

Using the above conditions the trifluoroethyl ester of carboxymolinate elutes at 7.7 minutes. See Figure 1 for typical chromatograms.

2. Calibration

Calibration solutions must be freshly prepared and derivatized concurrently with each set of aqueous samples to be analyzed. In order to avoid potential variations in extraction recovery, calibration solutions are not extracted from water. (Refer to discussion of solute matrix effects, Section II.F., below).

Working solutions of carboxymolinate in acetonitrile (section II.B.7) are first diluted by injecting a known aliquot into dichloromethane contained in a 50-mL centrifuge tube. The solvent is evaporated under vacuum, and the dried residue is derivatized as in Section II.C.3. Aliqouts are chosen such that the final concentration of derivatized product in toluene is comparable to the residue expected from aqueous samples. Refer to the table below for typical aliquots and final concentration values:

Syringe Capacity	Acetone * Back-Flush	Calibration Soln. Volume	Conc.	Weight Added to C-tube
50 µL	15 µL	25 µL of	1000 µg/mL	25.0 µg
50 µL	15 µL	25 µL of	100 µg/mL	2.50 µg
50 µL	15 µL	25 µL of	10 µg/mL	0.250 µg

* Note: the acetone is loaded into the microliter syringe, followed by the calibration solution; when the syringe contents are injected, the acetone effectively flushes the syringe needle, improving the reproducibility of syringe delivery.

A 25.0 µg aliquot of carboxymolinate is equivalent to the extract from an aqueous sample containing 25 µg per 25 mL of aqueous volume (eg., 1.0 ppm). The smaller aliquots are equivalent to 0.10 and 0.01 ppm carboxymolinate in water. All three concentrations are used to verify linearity of the method. The solution containing 2.5 µg carboxymolinate is used as a primary calibration solution.

3. Analysis of Extracts

Inject 2-µL of the final toluene solution containing derivatized sample residue or derivatized calibration solution residue. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of retention times (within 0.03 min) with

those of the calibration chromatograms. Reinject the primary calibration solution after injection of every four sample extracts. Inject all three calibration solutions at the beginning, middle, and end of the chromatographic run. Calculate the concentration of carboxymolinate in the sample extract by comparing it to the closest standard (peak height) or by use of a standard curve (refer to Figure 2).

E. Interferences

No cleanup was required when this procedure was used as described. However, extractives from water or from the derivatization reagents could potentially contribute peaks with retention times coincident with or near those of the analyte. In addition, repeated injection of derivatized sample solutions may lead to enhancement or depression of detector response (see discussion below). For this reason, the GC system (inlet, column, and detector) should be "conditioned" by repeated injections of a typical derivatized sample solution immediately prior to starting the analytical run.

Satisfactory chromatographic resolution can usually be achieved with appropriate oven temperature manipulations or column selection (length, phase). If resolution cannot be achieved, an alternate ion can be monitored. Analyze extracts of untreated control samples to demonstrate the absence of interferences from sample matrices, solvents, and labware. The resolution provided by capillary columns combined with the selectivity afforded by selective-ion-monitoring should eliminate any problems of misidentification.

F. Solute Matrix Effects

In this procedure, hydrochloric acid is used to protonate the carboxymolinate anion, so that the organic acid may be partitioned from water into a polar organic solvent. Sodium chloride is used to increase the partition coefficient (the aqueous solubility of the free acid form of the analyte decreases with increasing ionic strength). Any salts which partition into the organic solvent (or from carry-over during transfer between centrifuge tubes) may affect the chemical composition of the final toluene solution which contains derivatized products. Since the surface chemistry of the GC inlet, column, and detector can be "activated" (made less inert) or "deactivated" (made more inert) by trace amounts of chemical solutes, detector response can be altered by such sample "matrix effects".

If analyte recovery is outside acceptable limits for aqueous samples, it may be necessary to "matrix-match" the calibration solutions to achieve comparable detector response. For example, calibration solutions may be prepared by first extracting deionized water (with salt, acid, and dichloromethane, as in section II.C.1, above), then adding carboxymolinate working solution to the dichloromethane extract and proceeding with the evaporation and derivatization steps.

G. Confirmatory Techniques

Unexpected positive results, as in untreated control or pre-application samples, should be confirmed by other means. Confirmation can be achieved by quantitation using a different m/z ion or by using a different detector type, such as a nitrogen/phosphorus thermionic detector. Figure 3 shows mass-spectral scans for carboxymolinate and molinate, and a total ion chromatogram for carboxymolinate. A list of alternate m/z ions for carboxymolinate is given below:

	m/z	% relative abundance
	299	3
	200	5
	173	2
*	126	100

* Note: Base peak for both molinate and carboxymolinate; in SIM-mode, m/z 299 offers maximum selectivity for carboxymolinate.

H. Calculations

The concentration of the analyte in the original sample is calculated by using the external standard method, i.e., the response obtained for the analyte in the sample extract is compared to the response obtained from a separate injection of a known amount of analyte (calibration solution). If the response is linear, a factor can be calculated as described in II.H.1 below. If the response is non-linear, or if the analyst prefers, the analyte responses over a range of calibration solution concentrations can be fit to a linear or an exponential curve, and a factor can then be calculated as in II.H.2 below for each point on the curve that corresponds to an analyte response in an injection of sample extract. It is assumed for the calculations

outlined below that the injection volumes for all calibration solutions and sample extracts are fixed at the same volume.

1. Linear Detector-Response

   a. Calibration Factor

   Calculate the response factor, F, for injection of a calibration solution as follows:

$$F\ ((\mu g/mL)/\text{response unit}) = \frac{C_{std}}{R_{std} \times S}$$

   where C_{std} = concentration of calibration solution, µg/mL
   R_{std} = response units (e.g., peak height, peak area, electronic units) from detector for calibration solution
   S = ratio of amount (mL) of sample extracted to final volume (mL) of toluene (containing the derivatized product)

   b. Analyte in Sample

   Calculate the analyte concentration, R, in the original sample as follows:

$$R\ (\mu g/mL \text{ or ppm}) = F \times R_{sample}$$

   where F = response factor, ((µg/mL)/response unit)
   R_{sample} = response units from detector for analyte in the sample extract

   Averaged response factors obtained from injections of calibration solution before and after injection of sample extracts may be used for calculation of the analyte concentration in the sample.

2. Curve Fit for Linear or Non-linear Response

   If the instrumental response to injections of calibration solutions is reproducible and either linear or exponentially non-linear, a concentration-response curve can be used for sample quantitation. Any valid curve-fitting program can be used. Input

the concentration and response for each injection of calibration solution. The program will generate the formula for the corresponding linear or exponential curve (refer to Figure 2). From the formula, determine the calculated concentration for each injection of calibration solution as described below. For concentrations greater than ten times the detection limit, the calculated and actual concentrations should agree within 10% relative; that is, the ratio of the actual to the calculated concentration should be between 0.9 and 1.1. For concentrations at the detection limit, the agreement should be within 30% relative. If the agreement is adequate, calculate the concentration of analyte in the sample, and corresponding response factor as follows:

a. Linear Response

The formula will be of form $Y = mX + b$, where

Y = the concentration of the analyte, µg/mL

X = the detector response, peak height or area units,

and,

m and b = constants calculated by the curve-fit program.

Since the analyte concentration should be zero if the response is zero, the constant b should be zero if there are no systematic errors in the analysis. However, it is not necessary for b to be zero for the calculational method to be valid, as long as calibration solution responses are reproducible and the calculated concentrations of the calibration solutions are within acceptable limits.

For each sample injection, determine Y (µg/mL in the injected extract) by using the response, X, in the formula. Calculate the analyte concentration, R, in the original sample as follows:

$$R\ (\mu g/mL \text{ or } ppm) = Y/S$$

where S = ratio of amount (mL) of sample extracted to final volume (mL) of toluene (containing the derivatized product).

Calculate the response factor, F, from the formula:

$$F = R/X$$

b. Exponential non-linear response:

The curve will be of form $Y = aX^b$, where

Y = the concentration of the analyte, µg/mL

X = the analyte response, peak height or area units,

and

a and b = constants calculated by the curve-fit program.

For each sample injection, determine Y by using the response, X, in the formula. Calculate the analyte concentration, R, in the original sample as follows:

$$R\ (\mu g/mL \text{ or } ppm) = Y/S$$

Where S = ratio of amount (mL) of sample extracted to final volume (mL) of toluene (containing the derivatized product).

Calculate the response factor, F, from the formula:

$$F = R/X$$

The response factor will be different for each point on the curve.

C. Safety Plan

Personnel untrained in the routine safe-handling of chemicals and good laboratory practices must not attempt to use this procedure. Information on any specific chemical regarding physical properties, hazards, toxicity, and first-aid procedures can be found on the Material Safety Data Sheets accompanying the chemical or available from the chemical supplier. In general, always wear safety glasses with sideshields, work in a well

ventilated area, avoid inhaling vapors, and avoid contact of the chemical with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

1. Acetone. Flash point = 0°C; NIOSH Threshold Limit value (TLV) = 750 ppm.

2. Acetonitrile. Toxic and corrosive gases are formed if the solvent is heated to decomposition or ignited. Flash point = 42°C; NIOSH TLV = 40 ppm.

3. Hydrochloric Acid, (conc., 37% wt). 100 ppm is immediately hazardous to health. Causes severe burns and irritation. Remove contaminated clothing and wash affected skin or eyes with plenty of water after any accidental contact.

4. Dichloromethane. Toxic and corrosive gases are formed if the solvent is heated to decomposition. NIOSH TLV = 100 ppm.

5. Toluene. Flammable. Irritant. Flash point = 2°C; NIOSH TLV = 200 ppm.

6. Trifluoroacetic anhydride. Corrosive. Moisture-sensitive. Toxic and corrosive gases are formed if the solvent is heated to decomposition.

7. Trifluoroethanol. Flammable. Toxic. Severe irritant. Toxic and corrosive gases are formed if the solvent is ignited. Flash point = 29°C.

8. Carboxymolinate. Remove contaminated clothing and wash affected skin or eyes with plenty of water after any accidental contact.

IV. CONCLUSIONS

This method is specific for the analysis of carboxymolinate in aqueous samples. Only commercially available laboratory equipment and reagents are required. The analysis can be completed by one person in an 8-hr period. Untreated and fortified control samples should be extracted and analyzed with each set of samples to demonstrate absence of interferences and adequate recovery. If determination of carboxymolinate at a concentration other than 0.01 to 3.0 ppm is required, suitably fortified samples must be analyzed to validate the method at that concentration.