

## INTRODUCTION

This report contains validation data for analytical method GPL-MTH-076 (Revision 1) for the determination of MGK-264 in drinking water and surface water. The method was found to be suitable for the determination of residues of MGK-264 in drinking water and surface water over the concentration range of 0.05 to 1.0 ng/mL. The method LOQ was confirmed to be 0.05 ng/mL. The study was conducted to satisfy the requirements of US EPA OCSP 850.6100. The study protocol with analytical method appears in Appendix A.

ANALYTICAL

Storage and Characterization of Control Specimens

The control specimens, drinking water and surface water, were collected by EPL. Drinking water (ca. 4 L) was collected from a tap at EPL. Surface water (ca. 4 L) was collected from Lake Decatur, Decatur, IL. Control specimens were placed in a refrigerator when not needed in the laboratory. Each control specimen was characterized for various chemical parameters by AGVISE Laboratories under EPL study number 184G1131 in compliance with US EPA GLP Standards. Certificates of analysis for each control water specimen appear in Appendix B.

Preparation of Stock Solutions, Calibration Standard Solutions and Fortification Solutions

The primary reference item (analytical standard) certificate of analysis can be found in Appendix C. Reagents used were of equivalent specifications as those described in the analytical method.

The following primary reference item was utilized during the independent laboratory method validation.

Reference Item/Analytical Standard:	MGK-264
Chemical Name (CAS):	n-Octyl bicycloheptene dicarboximide
Supplier:	MGK
CAS No.:	000113-48-4
Batch/Lot No:	AB4552
Purity:	95.2%
Expiration date:	08/10/2016
Storage:	Room Temperature

Standard stock solutions, calibration standard solutions and fortification solutions were prepared as described in the analytical method. All standard solutions were stored refrigerated in amber glass bottles (ca. 4°C) when not needed in the laboratory. Full details of these materials were included in the raw data package for the study along with details of the preparation of all analytical and fortification standards prepared from the primary reference item. The reference item will be retained until expiration and then disposed of with Sponsor approval following relevant EPL Standard Operating Procedures.

Fortification of Recovery Samples

The control specimens were fortified as described in the table below.

Matrix	Reference Item	Unfortified Control Specimens	Replicates at Fortification Level LOQ	Replicates at Upper Fortification Level 20x LOQ
Drinking Water	MGK-264	2	7 at 0.05 ng/mL	7 at 1 ng/mL
Surface Water	MGK-264	2	7 at 0.05 ng/mL	7 at 1 ng/mL

Method Principle

Test portion extracts were assayed according to the analytical method appearing in Appendix A. The method is applicable to the determination of MGK-264 in drinking water and surface water using HPLC-MS/MS.

Sample Preparation for Analysis

1. Five (5.0) mL aliquots of untreated control water were pipetted into glass vials.

For recovery samples, an appropriate volume of spiking solution was added to obtain concentrations ranging from 0.05 ng/mL to 1.0 ng/mL as described in the table below:

Description	Spiking Volume (mL)	Spiking Solution conc. (ng/mL)	Fortification Level (ng/mL)
Control	---	---	---
LOQ	0.1	2.5	0.05
20x LOQ	0.1	50	1.0

2. After fortification, 5.0 mL of acetonitrile (ACN) were combined with the sample.
3. Samples were filtered using a 0.45 µm PTFE syringe filter into a clean glass vial.
4. From the filtered extract, an aliquot was transferred to an HPLC autosampler vial and analyzed by HPLC-MS/MS.

Analytical Instrumentation and Equipment

Chromatographic Conditions

Instrumentation: Agilent 1290 Infinity Series  
HPLC Column: Luna C18, 30 mm x 2.0 mm x 3 µm, Phenomenex  
Column Oven Temperature: Ambient  
Data System: Analyst version 1.6, AB SCIEX  
Injection Volume: 20 µL

Eluent A: 0.2% Formic Acid in ACN  
Eluent B: 0.2% Formic Acid in DI Water

Gradient:

Time (minutes)	% Eluent A	% Eluent B	Flow (µL/minute)
0.0	50	50	500
3.4	80	20	500
3.5	50	50	500
5.0	50	50	500

MGK-264 Retention Times: ca. 1.8 and 2.2 minutes (2 isomers)

Mass Spectrometric Conditions

MS System: TRIPLE QUAD 6500, AB SCIEX  
 Ionization Source: Electrospray Ionization (ESI)  
 Polarity: Positive  
 Scan Type: Tandem MS (MS/MS), Multiple Reaction Monitoring (MRM)  
 Capillary Voltage: 5500 V  
 Curtain Gas (CUR): 30 Gas Flow 1 (GS1): 40  
 Collision Gas (CAD): 6 Gas Flow 2 (GS2): 40

Analyte Monitored	Mass Transition Monitored (m/z)	Declustering Potential (DP) (V)	Entrance Potential (EP) (V)	Collision Energy (CE) (V)	Cell Exit Potential (CXP) (V)	Dwell Time (ms)
MGK-264	276.2→210.1 <sup>1</sup>	80	12	21	15	100
	276.2→98.0	80	12	34	15	300

<sup>1</sup> Proposed quantitative transition. Both transitions were used to obtain quantitative data for this study.

Calculation of Results

Calibration standard solutions were injected over the concentration range of 0.005 - 1 ng/mL. The peak area responses of the two isomers of MGK-264 were computed using Multiquant software. The peak area responses of the two isomers were then summed using Microsoft Excel. A calibration curve was constructed using the summed peak area response and the standard concentration using the Multiquant (version 2.1, AB SCIEX) Metric Plots function. A weighting factor of 1/x was used. Sample solution concentrations were calculated using the straight line equation from the calibration curve.

Example Calculations

Values appearing in raw data tables (Appendix D) may differ slightly due to rounding. The examples provided are for the proposed quantitative MRM transition (m/z 276 → 210). The same calculations apply to the proposed confirmatory transition (m/z 276 → 98).

MGK-264 recovery at 0.05 ng/mL in Drinking Water (LOQ fortification level):

Laboratory ID: 1131-D001-S1, Set V001

Total MGK-264 Peak Area: 15346 (Isomer 1 = 7364, Isomer 2 = 7982, Sum = 15346)

Regression Equation:  $y = 610568 * x - 309.45280$

Where: y = peak area  
x = concentration (ng/mL)

$$MGK-264 \text{ (ng/mL)} = (15346 + 309.45280) / 610568 = 0.0256$$

*Measured Residue (ng/mL MGK-264) =*

$$\frac{\text{MGK-264 (ng/mL)} * \text{Total Volume (mL)}}{\text{Sample Amount (mL)}}$$

MGK-264 (ng/mL): 0.0256  
Total Volume (mL): 10  
Sample amount (mL): 5

*Measured Residue (ng/mL MGK-264) =*

$$\frac{0.0256 \text{ ng/mL} * 10 \text{ mL}}{5 \text{ mL}} = 0.0512$$

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*Fortification Amount (ng/mL) =*

$$\frac{\text{Volume of Spiking Solution (mL)} * \text{Spiking Solution Concentration (ng/mL)}}{\text{Sample Amount (mL)}}$$

Volume of Spiking Solution (mL): 0.1  
Spiking Solution Concentration (ng/mL): 2.504  
Sample Amount (mL): 5

*Fortification Amount (ng/mL) = 0.1 mL \* 2.504 ng/mL / 5 mL = 0.0501*

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*Recovery (%) =*

$$\frac{\text{Measured Residue, Fortified Sample (ng/mL)} - \text{Measured Residue, Control Sample (ng/mL)} * 100}{\text{Fortification Amount (ng/mL)}}$$

Measured Residue Fortified Sample (ng/mL): 0.0512  
Measured Residue Control Sample (ng/mL): 0.0032 (mean of duplicates)  
Fortification Amount (ng/mL): 0.0501

*Recovery (%) = (0.0512 ng/mL - 0.0032 ng/mL) / 0.0501 ng/mL \* 100 = 95.8%*

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#### Statistical Treatment of Data


The mean recoveries for the fortified samples were calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries was calculated using the "STDEV" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. RSD (%) values were calculated by dividing the standard deviation by the mean, and then multiplying by 100.

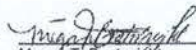


Analytical Procedure for the Determination of MGK-264 in Water

GPL-MTH-076 (Revision 1)  
Effective April 14, 2014

Reason for revision: Incorporate new lower LOQ and surface water matrix.

Prepared by:  05/02/14  
Date  
Kimon P. Acedo  
Chemist  
Golden Pacific Laboratories, LLC

Reviewed by:  May 2, 2014  
Date  
Megan T. Boatwright  
Lab Manager  
Golden Pacific Laboratories, LLC

## 1.0 INTRODUCTION

MGK-264 is used as an insecticide synergist on multiple pest types. An analytical procedure is required for the determination of residues of MGK-264. This method is concerned with the measurement of MGK-264 residues in tap or surface water.

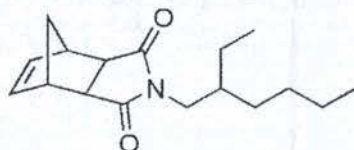
MGK-264, when analyzed by LC-MS/MS resolves into two isomer peaks (with the same ion pairing) at different retention times. The peak areas of the two peaks are summed to determine the total amount of MGK-264 in a standard or sample.

The target limit of quantitation (LOQ) and the limit of detection (LOD) for MGK-264 in water are 0.0500 ng/mL and 0.0170 ng/mL, respectively.

## 2.0 REFERENCE SUBSTANCE

McLaughlin Gormley King Company, USA, provided the reference substance, MGK-264. The reference substance is used to prepare calibration and fortification solutions, and to determine procedural recoveries.

### 2.1 REFERENCE SUBSTANCE



Common Name: MGK-264 (n-Octyl bicycloheptene dicarboximide)  
CAS No.: 000113-48-4  
Source: McLaughlin Gormley King Company

A copy of the certificate of analysis of the reference substance will be kept in the archives at GPL. The reference substance will be stored refrigerator set to maintain  $4\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  (refrigerated).

## 3.0 PRINCIPLE OF THE METHOD

A 5-mL aliquot of the representative water sample is combined with 5 mL of acetonitrile. After the acetonitrile is added, the sample is shaken by hand and filtered. It is then analyzed for MGK-264 residues using LC-MS/MS.

Six concentrations of the reference substance in 50% acetonitrile: 50% HPLC Grade water are used for standards. A calibration plot is drawn for MGK-264 and used for quantitation purposes.

#### 4.0 EQUIPMENT AND MATERIALS

Unless otherwise indicated, the equipment and materials listed below may be substituted with functionally equivalent equipment.

- Balance, Analytical: Mettler model AB 204-S, precision  $\pm 0.1$  mg, for standards
- Balance, Analytical: Mettler model PB3002-S, precision  $\pm 0.01$  g, for samples
- Disposable Pasteur pipettes, glass
- Jar, amber glass: with Teflon lined cap, 30 mL, 60 mL, 120 mL
- Test tube or vial with Teflon lined cap, glass, various sizes
- VWR Syringe Driven Filters (PTFE) 0.45- $\mu$ m
- Thermo Scientific 10-mL plastic disposable syringes
- Wiretrol pipette: 100  $\mu$ L
- Volumetric flask, glass: 25 mL
- Volumetric pipette, various sizes
- HPLC vials, clear glass: 1.8 mL
- AB Sciex API4000 LC-MS/MS with Shimadzu LC-20AD HPLC Pumps, Shimadzu SCL-10A VP Controller, and Shimadzu SIL-20AC Autosampler or Shimadzu SIL-20AC HT Autosampler with Analyst Software
- AB Sciex API5000 LC-MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, and Shimadzu SIL-20AC XR Autosampler with Analyst Software

#### 5.0 CHEMICALS/REAGENTS

Alternate suppliers of reagents having comparable specifications may be used.

- Acetonitrile, Fisher #A996-4
- Water, HPLC Grade, VWR #MK679510
- Formic Acid, 88%, Fisher #A118P-500

#### 6.0 STANDARD SOLUTIONS

The reference substance is used in the preparation of the fortification and calibration solutions. The reference substance is stored refrigerated.

##### 6.1 MGK-264 Fortification Solutions

The MGK-264 reference substance is first weighed directly into a 25-mL volumetric flask and made up to volume with acetonitrile to give a solution with a concentration of approximately 1.0 mg/mL MGK-264 (Solution A) after being corrected for purity.



An aliquot (5.0 mL) of Solution A (1.0 mg/mL) is diluted with acetonitrile to a final volume of 20 mL to prepare an intermediate solution of approximately 250 µg/mL (Solution B). A 25 µg/mL fortification solution (Solution C) is prepared by taking a 5.0-mL aliquot of Solution B (250 µg/mL) and diluting it to a final volume of 50 mL. From Solution C, a second fortification solution is prepared by taking an aliquot of 2.0-mL and diluting to a final volume of 50 mL (Solution D with a concentration of 1.0 µg/mL). Finally a 50 ng/mL solution (Solution E) is prepared by taking a 5.0-mL aliquot of Solution D (1.0 µg/mL) and diluting it to a final volume of 100 mL.

Separate fortification solutions may also be prepared to achieve lower fortification levels. An aliquot (0.1 mL) of Solution A (1.0 mg/mL) is diluted with acetonitrile to a final volume of 100 mL to give a solution with a concentration of approximately 1.0 µg/mL (Solution F). A 50 ng/mL fortification solution (Solution G) is prepared by taking a 2.0-mL aliquot of Solution F (1.0 µg/mL) and diluting with acetonitrile to a final volume of 40 mL. A 5.0 ng/mL fortification solution (Solution H) is prepared by taking a 0.1-mL aliquot of Solution F (1.0 µg/mL) and diluting with acetonitrile to a final volume of 20 mL.

The fortification solutions are stored refrigerated in amber bottles and renewed every 3 months or as needed.

#### 6.2 Calibration Solutions

Solution E will be used to make calibration standards at appropriate concentrations. Calibration standards are made in 50% acetonitrile: 50% water. Typical concentrations are 10 ng/mL, 5.0 ng/mL, 2.0 ng/mL, 1.0 ng/mL, 0.50 ng/mL, and 0.25 ng/mL.

Separate calibration solutions may also be prepared to analyze lower water concentrations using the API5000 instrument. Solution H is used to make calibration solutions diluted with acetonitrile: water (50: 50, v/v). Typical concentrations are 1.0 ng/mL, 0.50 ng/mL, 0.10 ng/mL, 0.050 ng/mL, 0.025 ng/mL and 0.0125 ng/mL.

All calibration solutions are stored refrigerated in amber bottles. Calibration solutions are prepared every three months or as required.

#### 7.0 ANALYTICAL PROCEDURE

A 5-mL sample of water is pipetted into a glass test tube or glass vial and fortified at the appropriate concentration. The targeted levels are as follows:

	Fortification Levels
LOQ (API5000)	0.05 ng/mL
LOQ (API4000)	1.00 ng/mL
MID	20.0 ng/mL
HIGH	500 ng/mL

Laboratory fortifications are prepared using a syringe or Wiretrol pipette.

After fortification, 5 mL of acetonitrile is combined with the sample. Note: If the protocol dictates that the sample results should be reported in ng/g or ppb, 5 mL of the sample is weighed out into the glass test tube or glass vial (this sample weight is recorded) and then 5 mL of acetonitrile is added. Samples are gently shaken by hand. Next, the sample is filtered through a disposable plastic syringe with a PTFE 0.45- $\mu$ m filter into a new glass test tube or glass vial. From the filtered extract, an aliquot is transferred to a chromatography vial and analyzed by LC-MS/MS. Samples having higher residue levels are diluted to an appropriate final volume using 50% acetonitrile: 50% water so that the response falls within the calibration range of the standards.

## 8.0 QUANTITATION

### 8.1 Instrumentation

An AB Sciex API4000 LC-MS/MS system is used. The HPLC consists of Shimadzu LC-20AD HPLC Pumps, a Shimadzu SCL-10A VP Controller, and Shimadzu SIL-20AC Autosampler or Shimadzu SIL-20AC HT Autosampler. Data is acquired using Analyst Software.

For surface ground water and tap water analysis requiring a lower LOQ (0.05 ng/mL) an AB Sciex API5000 LC-MS/MS system is used. The HPLC consists of Shimadzu LC-20AD XR Pumps, a Shimadzu CBM-20A Controller, and Shimadzu SIL-20AC XR Autosampler. Data is acquired using Analyst Software.

### 8.2 HPLC Conditions

**Analytical Column:** Phenomenex, Luna 3- $\mu$  C18 100A  
30 x 2.00 mm

**Column Temperature:** ambient

**Mobile Phase:** Gradient:  
A% = 0.2% formic acid in acetonitrile  
B% = 0.2% formic acid in water

Time (min)	A (%)	B (%)
0.0	50.0	50.0
3.0	80.0	20.0
3.1	50.0	50.0
4.5	50.0	50.0

For the API5000 instrument with reduced LOQ, the following mobile phase gradient is employed:

Time (min)	A (%)	B (%)
0.0	50.0	50.0
3.4	80.0	20.0
3.5	50.0	50.0
5.0	50.0	50.0

**Flow Rate:** 500 µL/min  
**Injector:** autosampler  
**Injection Volume:** 10 µL (1.0 ng/mL LOQ)  
20 µL (0.05 ng/mL LOQ)

**Approximate Retention Times for the Two Resolved Isomers:** 2.2 and 2.5 minutes

### 8.3 Mass Spectrometer Parameters

For the API4000 the following Mass Spectrometer Parameters are used:

**Interface:** TurbolonSpray (ESI)  
**Polarity:** Positive  
**Scan Type:** MRM Monitoring with Unit/Low resolution

**Curtain Gas (CUR):** 20.0  
**(GS1):** 40.0  
**(GS2):** 40.0  
**Ionspray Voltage (IS):** 5500  
**Temperature (TEM):** 450 °C  
**Collision Gas (CAD):** 6.00  
**Entrance Potential (EP):** 10.0  
**Declustering Potential (DP):** 61.0  
**Collision Energy (CE):** 20.0 (Quant.) 34.0 (Conf)  
**Collision Cell Exit Potential (CXP):** 10.0

For the API5000, the following Mass Spectrometer Parameters are used:

Interface:	TurboIonSpray (ESI)	
Polarity:	Positive	
Scan Type:	MRM Monitoring with Unit/Low resolution	
Curtain Gas (CUR):	30.0	
(GS1):	40.0	
(GS2):	40.0	
Ionspray Voltage (IS):	5500	
Temperature (TEM):	450 °C	
Collision Gas (CAD):	6.00	
Entrance Potential (EP):	12.0	
Declustering Potential (DP):	80.0	
Collision Energy (CE):	21.0 (Quant )	34.0 (Conf )
Collision Cell Exit Potential (CXP):	15.0	

Ions Monitored:

MGK-264 (Quantitation)	Q1 276.2 m/z
	Q3 210.1 m/z
MGK-264 (Confirmation)	Q1 276.2 m/z
	Q3 98.0 m/z

#### 8.4 LC-MS/MS Detector Response Calibration

The peak area responses for the two isomers of MGK-264 are computed using the Analyst software. The peak area responses of the two isomers are summed using Analyst's formula columns. This summed value represents the peak area response for total MGK-264. See Equation 1.

For the standards, a curve using the peak area response for total MGK-264 and the concentrations of the calibration standards is generated using Analyst's Metric Plots capability. The concentrations of the standards in ng/mL are plotted as the X-axis. The summed peak area responses are plotted as the Y-axis to give Equation 2.

Peak area of total MGK-264 =

*peak area of isomer RT 2.1 min + peak area of isomer RT 2.4 min*

[Eq. 1]

$$y = mx + b \quad [\text{Eq. 2}]$$

where:

$y$  = summed peak area response (peak area of total MGK-264)

$m$  = slope of the regression line

$x$  = amount (ng/mL) of analyte found in the standard

$b$  = intercept of the regression line

$$\text{total peak area} = m (\text{ng/mL in the standard}) + b \quad [\text{Eq. 2}]$$

#### 8.5 Sample Analysis

The amount of MGK-264 in a sample is determined from the corresponding standard calibration plot. Concentration of MGK-264 in ng/mL is calculated from the summed peak area using Equation 3.

$$x (\text{ng MGK-264/mL}) = \frac{\text{total peak area of MGK-264} - b}{m} \quad [\text{Eq. 3}]$$

Any apparent residues found in the control samples, greater than 30% of the lowest standard injected, are subtracted as raw peak area from the fortified samples.

Both samples and standards must be analyzed under the same LC-MS/MS conditions and within the same analytical sequence.

#### 9.0 CALCULATION OF RESIDUES

From the standard calibration curve, MGK-264 concentrations (ng/mL or ng/g) in unknown samples are determined using the following equation:

$$\text{ng/mL} = \frac{(\text{ng/ml from curve}) (\text{total volume in mL})}{(\text{sample amount (mL)})} \quad [\text{Eq. 4}]$$

$$\% \text{ Recovery} = \frac{\text{measured residues (ng/mL)}}{\text{fortification amount (ng/mL)}} \times 100 \quad [\text{Eq. 5}]$$

#### 10.0 QUALITY CONTROL PROCEDURES

##### 10.1 LC-MS/MS Analysis

The calibration standards from the analytical set are plotted and the standard linear regression, slope and y-intercept values are calculated using a  $1/x$  weighted curve. These values are used to establish the fortification recoveries. The calibration plot is not forced through zero.

There are a minimum of five calibration standards that bracket the concentration range of interest with the lowest standard corresponding to 70% or less of the lowest level of interest. Sample extracts containing analyte levels outside this range are diluted accordingly to fit the calibrated range. Calibration standards are injected at the beginning and end of an analytical set. Standards are also injected periodically throughout the set. No more than six sample injections are made without a standard injection.

#### 10.1.1 Acceptance Criteria

The acceptance criterion for the correlation coefficient ( $r$ ) of the calibration curve must be  $\geq 0.990$  (or consequently, the coefficient of determination ( $r^2$ ) must be  $\geq 0.980$ ).

The acceptance criteria for the average QC recovery are 70 – 120%. The acceptance criteria for the lowest calibration standard (at or below the LOQ) will be an RPD value of  $\leq 25\%$  and  $\leq 20\%$  for all other calibration standards.

Acceptance criterion for control samples (unfortified, blank matrix samples) will be  $\leq 30\%$  of the LOQ as estimated from peak area response.

Any contribution from the control samples that is  $> 30\%$  of the LOQ at the retention time of the analytes may be subtracted from the response of the fortified sample as raw peak area. When the peak area in a control is  $\leq 30\%$  of the lowest standard injected, the contribution will be considered negligible at the discretion of the Study Director.

#### 11.0 QUANTITATION LIMIT

The limit of quantitation (LOQ) for this method is 1.00 ng/mL using an API4000 instrument and 0.05 ng/mL using an API5000 instrument.

12.0 METHOD FLOW CHART

