

1.0 INTRODUCTION

Analytical procedure GPL-MTH-076 was developed and successfully validated for the determination of residues of MGK-264 in surface ground water and tap water. The original limit of quantitation (LOQ) for MGK-264 in water was 1.0 ng/mL. In 2014, the study was re-opened by amendment to add an extra validation set showing the method was successful with a reduced LOQ of 0.05 ng/mL in tap water and surface ground water. This amended final report includes the additional data at the lower LOQ, as well as the original validation data.

The analytical method was validated using sample extracts, fortification solutions and calibration standards.

2.0 PURPOSE

The study was designed to validate GPL Analytical Method GPL-MTH-076 "Analytical Procedure for the Determination of MGK-264 in Water." This method was validated for MGK-264 in tap water using LC-MS/MS.

2.1 Method Validation Design

Control samples of tap water were fortified with known amounts of MGK-264 in acetonitrile and analyzed using GPL Analytical Method GPL-MTH-076. Three control and twenty-one fortified control samples (seven at 3 fortification levels, LOQ, a mid-fortification level and a high fortification level) were prepared. Each sample was assigned a unique number using an alphanumeric code, where:

TW = Tap Water

C1-C3 = non-fortified controls

F1-1 to F1-7 = fortified level corresponding to LOQ, replicates 1 - 7

F2-1 to F2-7 = fortified level corresponding to the mid-fortification level, replicates 1 - 7

F3-1 to F3-7 = fortified level corresponding to the high fortification level, replicates 1 - 7

The levels of fortification were LOQ, 20X and 500X LOQ.

Matrix	Fortification Levels		
	MGK-264 Low Level	MGK-264 Mid Level	MGK-264 High Level
Tap Water (5 mL)	1.00 ng/mL	20.0 ng/mL	500 ng/mL

2.2 Amended Method Validation Design

The study was re-opened after finalization to amend the report and validate surface ground water and tap water at lower fortification levels. Control samples of surface ground water and tap water were fortified with known amounts of MGK-264 in acetonitrile and analyzed using GPL Analytical Method GPL-MTH-076. Two control and fourteen fortified control surface ground water samples (seven at 2 fortification levels, LOQ and 10X LOQ) were prepared. Two control and seven fortified control tap water samples fortified at the LOQ were prepared. Each sample was assigned a unique number using an alphanumeric code, where:

TW = Tap Water
SGW = Surface Ground Water

C1 and C2 = non - fortified controls
F4-1 to F4-7 = fortified level corresponding to LOQ, replicates 1 - 7
F5-1 to F5-7 = fortified level corresponding to the 10X LOQ fortification level, replicates 1 - 7

Matrix	Fortification Levels	
	Amended MGK-264 Low Level (LOQ)	Amended MGK-264 Mid Level (10X LOQ)
Tap Water	0.05 ng/mL	NA
Surface Ground Water	0.05 ng/mL	0.5 ng/mL

3.0 REFERENCE SUBSTANCE

The reference substance was MGK® 264 Insecticide Synergist Technical (MGK-264). The MGK-264 reference substance was used for the preparation of the calibration solutions and fortification solutions used to fortify the tap water. The reference substance was stored at in a refrigerator set to maintain $4\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ (refrigerated).

3.1 Reference Substance Specifications

The reference substance, MGK® 264 Insecticide Synergist Technical (MGK-264), was supplied by McLaughlin Gormley King Company.

	Original Work	Amended Work
Name	MGK® 264 Insecticide Synergist Technical (MGK-264)	
CAS Number	000113-48-4	
Active Ingredient	MGK-264	
Lot Number	AB1409	AB4552
Purity	96.3%	95.2%
Date Received	March 07, 2011	January 23, 2014
Expiration	March 03, 2012	January 22, 2015

A copy of the certificates of analysis of the reference substances will be kept in the study records, and also in the facility archives at GPL.

4.0 EQUIPMENT AND MATERIALS

The equipment and materials used are listed below:

- Balance, Analytical: Mettler model AB 204-S, precision ± 0.1 mg, for standards
- Balance, Analytical: Mettler model PB3002-S, precision ± 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 μ m
- Thermo Scientific 10-mL plastic disposable syringes
- Wiretrol pipette: 100 μ 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 version 1.5.2
- 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 version 1.5.2

5.0 CHEMICALS/REAGENTS

The following chemicals, reagents and supplies were used:

- Acetonitrile, Optima Grade, Fisher #A996-4
- Water, HPLC Grade, VWR# MK679510
- Water, HPLC Grade, Fisher #W5-4
- Formic Acid, 88%, Certified ACS, Fisher #A118P-500

6.0 TEST SYSTEM

The test system for this study was tap water collected at the analytical laboratory and surface ground water collected from a nearby river.

6.1 Tap Water

The drinking (tap) water control matrix was obtained from the Fresno municipal supply at GPL on 04/01/2014 and stored refrigerated. The characterization of the Fresno municipal supply water is presented in the Water Quality Report located in Appendix 4.

6.2 Surface Ground Water

The surface water control matrix was obtained from the San Joaquin River in Fresno, CA at a point near Gravel Haul Road on 03/27/2014. Sub-portions of this sample were labeled with sample number "SJR-032714" and were then transported by GPL personnel to BSK Laboratories in Fresno, California for non-GLP characterization. The non-GLP characterization results are located in Appendix 4 and summarized below:

Parameter	Found Value
pH	7.7
Dissolved Oxygen	11 mg/L
Conductivity	60 μ mhos/cm
Alkalinity	24 mg/L as CaCO ₃
Total Hardness	21 mg/L as CaCO ₃
Total Solids	40 mg/L
Total Organic Carbon	2.2 mg/L
Dissolved Organic Carbon	2.2 mg/L

Sub-portions from the sample "SJR-032714" (refrigerated after collection) were used for the amended portion of the method validation.

7.0 STANDARD SOLUTIONS

The MGK® 264 Insecticide Synergist Technical (MGK-264) reference substance was used in the preparation of the fortification and calibration solutions.

7.1 MGK-264 Fortification Solutions

Typical stock solutions were prepared by weighing 0.025 g of the reference substance directly into a 25-mL volumetric flask and bringing up to volume with acetonitrile (ACN). After correcting for purity, the stock solution contained nominally 1.0 mg/mL (Solution A).

A 5.0 mL aliquot of solution A was diluted with ACN to a final volume of 20 mL, resulting in a solution containing 250 µg/mL (Solution B).

A more dilute solution was prepared by taking a 5.0 mL aliquot of Solution B and diluting it to 50 mL with ACN, resulting in a solution having a concentration of 25 µg/mL (Solution C).

Another solution was prepared by taking a 2.0 mL aliquot of Solution C and diluting it to a final volume of 50 mL with ACN. This created Solution D resulting in a concentration of 1.0 µg/mL.

A 5.0 mL aliquot of Solution D was diluted to a final volume of 100 mL with ACN, resulting in a solution having a concentration of 50 ng/mL (Solution E).

Solutions C, D, and E were used as fortification solutions in the original method validation experiment to fortify samples at the higher LOQ (1.0 ng/mL).

For the amended portion of the study, separate lower concentration fortification solutions were prepared to achieve the lower LOQ level. A 0.1 mL aliquot of Solution A was diluted to a final volume of 100 mL with ACN, resulting in a solution having a concentration of 1.0 µg/mL (Solution F).

A 2.0 mL aliquot of Solution F was diluted to a final volume of 40 mL with ACN, resulting in a solution having a concentration of 50 ng/mL (Solution G).

A 0.1 mL aliquot of Solution F was diluted to a final volume of 20 mL with ACN resulting in a solution having a concentration of 5.0 ng/mL (Solution H).

Solutions G and H were used as fortification solutions in the amended portion of the study to fortify samples at the lower LOQ (0.05 ng/mL).

All fortification solutions were stored in amber bottles and refrigerated.

7.2 MGK-264 Calibration Solutions for LC-MS/MS Analysis

Aliquots of Solution E were taken with volumetric glass pipettes to make calibration standards for the analysis of the original validation portion of the study. Calibration standards were made up in acetonitrile: water (50:50, v/v). Typical concentrations of calibration standards are shown below:

Volume Used (mL)	Final Volume (mL)	Concentration (ng/mL)
10.0	50	10.0
5.0	50	5.00
5.0	100	2.50
2.0	100	1.00
1.0	100	0.500
0.5	100	0.250

For the amended portion of the study, a lower concentration calibration curve was prepared by diluting aliquots of Solution H with acetonitrile: water (50:50, v/v) as shown below:

Volume Used (mL)	Final Volume (mL)	Concentration (ng/mL)
10.0	50	1.00
5.0	50	0.500
2.0	100	0.100
1.0	100	0.0500
0.1	20	0.0250
0.1	40.1	0.0125

All calibration solutions were stored refrigerated in amber bottles.

8.0 ANALYTICAL METHOD

8.1 Principle of the Method

MGK-264 residues were determined by combining an aliquot of the sample with an equal volume of acetonitrile. The resulting extract was filtered, and analyzed using a LC-MS/MS method for MGK-264 residues.

Six concentrations of the reference substance were used for standards. Calibration plots were drawn for MGK-264 peak area versus concentration. Linear regression with 1/x weighting was used for quantitation purposes.

8.2 Analytical Procedure for Tap Water

Sample tap water aliquots (5-mL) were pipetted into 16-mL vials. Each fortified sample was fortified with solution B, C or D for the higher LOQ levels, or solution G and H for the lower LOQ levels. Fortification of all samples was performed using Wiretrol pipets. The samples were fortified at 1.00 ng/mL, 20 ng/mL and 500 ng/mL in the original portion

of the study. Samples were fortified at 0.05 ng/mL and 0.50 ng/mL for the amended portion of the study.

An aliquot, (5-mL) of acetonitrile was combined with the tap water sample in a 16-mL vial. The tubes were capped and gently shaken by hand. The samples were then filtered through a PTFE 0.45- μ m syringe filter. Sample extracts having higher residue levels are diluted to an appropriate final volume, using acetonitrile: water (50:50, v/v) so that the response falls within the calibration range of the standards. An aliquot of the final sample extract (or diluted extract) was transferred to a chromatography vial and was analyzed by LC-MS/MS.

8.3 Instrumentation

An AB Sciex API4000 or API5000 LC-MS/MS system was used as described in section 4.0. For surface ground water and tap water analysis requiring a lower LOQ (0.05 ng/mL) an AB Sciex API5000 LC-MS/MS system was used.

8.3.1 LC-MS/MS Conditions

Analytical Column: Phenomenex, Luna 3 μ
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

For the analysis using the API4000 instrument, the following mobile phase gradient was employed:

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 was 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)

Approx. Retention Times of MGK-264: ~2.2 and 2.5 minutes

8.3.2 Mass Spectrometer Parameters

Interface: TurbolonSpray® (ESI)

Polarity: Positive

Scan Type: MRM Monitoring with Unit/Low resolution

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

Instrument parameters were optimized to give the maximum sensitivity with the standards. The confirmation ion was monitored to confirm the specificity of the analysis method.

8.4 Quantitation

8.4.1 Detector Response Calibration

The peak area responses for the two isomer peaks of MGK-264 were computed using the Analyst software. The peak area responses of the two isomers were summed using Analyst's formula columns. This summed value represented 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 was generated using Analyst's Metric Plots capability. The concentrations of the standards in ng/mL were plotted as the X-axis. The summed peak area responses were plotted as the Y-axis to give Equation 2.

Peak area of total MGK-264 =

$$\text{peak area of isomer RT 2.1 min} + \text{peak area of isomer RT 2.4 min} \quad [\text{Eq. 1}]$$

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

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

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

8.4.2 Sample Analysis

For samples, the amount found (ng/mL) of MGK-264 was calculated from the observed total peak area response using Equation 3.

Peak area of total MGK-264 =

$$\text{peak area of isomer RT 2.1 min} + \text{peak area of isomer RT 2.4 min} \quad [\text{Eq. 1}]$$

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

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

9.0 CALCULATION OF RESIDUES AND RECOVERIES

From the standard calibration curve, MGK-264 concentrations (ng/mL) in fortified tap water samples were determined using the following equations:

$$\text{ng/mL} = \frac{(\text{ng/mL from curve} \times \text{final volume in mL})}{(\text{Sample volume (mL)})} \quad [\text{Eq. 4}]$$

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

9.1 Example Calculation of Residues and Recoveries

For tap water sample TW-F1-1, which was fortified with 1.00 ng/mL of MGK-264 (Chromatographic Data Sheet 375MV01-4, Appendix 3), the residues (ng/mL) found and the MGK-264 recoveries were calculated as follows:

Using Equation 3:

$$\text{ng/g of MGK-264 found} = \frac{[(17609.45) - (-693)]}{35500} = 0.516 \text{ ng/mL}$$

Using Equation 4:

$$\text{Residues} = \frac{0.516 \text{ ng/mL} * 10 \text{ mL}}{(5 \text{ mL})} = 1.03 \text{ ng/mL}$$

Using Equation 5:

$$\% \text{ Recovery} = \frac{1.03 \text{ ng/mL}}{1.00 \text{ ng/mL}} * 100 = 103\%$$

Minor variations in values between example calculation and data sheet originate from adjusting data to three significant figures.

9.2 Statistical Methods

The statistical methods were limited to calculating the mean, standard deviation and % CV of recovery values. Calibration curves were not forced through zero.