# 1.0 INTRODUCTION/SUMMARY

## 1.1 Scope

This method is used for the determination of Mesotrione [Chemical Abstracts Registry (CAS) Number: 104206-82-8, (2-(4-methylsulfonyl-2-nitriobenzoyl)-1,3-cyclohexanedione)] and its degradates AMBA, and MNBA in water. The compounds are separated by high performance liquid chromatography (HPLC) and detected by mass spectrometry (LC/MS). An Ion-Spray atmospheric pressure ionization (API) interface is used to introduce the HPLC effluent into the mass spectrometer. The analytes are detected in the triple quadrupole mode (MS/MS) by passing the positive/negative molecular ion through Q1, inducing fragmentation in Q2, and then monitoring a characteristic product ion in Q3. The chemical structures, chemical names, and Chemical Abstracts Registry numbers of the analytes are presented in Figure 1.

The limit of detection by LC/MS (smallest standard amount injected during the chromatographic run) is 1.25 pg for all analytes. The limit of determination (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for LC/MS analyses is 0.05 ppb for all analytes in water.

## 1.2 Principle

An aliquot of the sample is acidified and transferred into an HPLC autosampler vial for analysis by LC/MS.

# 2.0 MATERIALS AND METHODS

## 2.1 Apparatus

1) Balance, analytical (Sartorius R160P) or equivalent.

2) Pasteur pipette (Fisher cat. #13-678-7C) or equivalent.

3) Cylinder, graduated, 100-mL, 50-mL, 25-mL, and 10-mL (Fisher cat. #08-550E, 08-550D, 08-550C and 08-551B) or equivalent.

4) Pipettes, glass, class A certified, assorted volumes. These pipettes are used when an exact addition of liquid is required (i.e., dilution of standards).

5) Pipetters, Oxford BenchMate adjustable, 40-200 µL volume range (Fisher cat. #21-231), 200-1000 µL volume range (Fisher cat. #21-229) or equivalent. (Note: These adjustable pipetters may only be used for addition of liquid where an exact volume added is not critical, i.e., addition of acid or base.).

6) Vials, clear or amber, 1.5-mL (Sun Brokers, Inc. cat. #200-002) or equivalent, with Teflon-lined, crimp-top seals (Sun Brokers, Inc. cat. #200-152) or equivalent.

7) Polypropylene 15-mL centrifuge tube (VWR, cat #20171-012) or equivalent

## 2.2 Reagents and Analytical Standards

All reagents are stored at room temperature. Solid analytical standards are stored in a freezer (temperature <-10°C).

1) Acetic acid, HPLC grade (Fisher cat. #A35-500) or equivalent.

2) Acetic acid, 0.1% solution: Mix 1 mL of acetic acid with 999 mL of purified water.

3) Acetonitrile, HPLC grade (Fisher cat. #A998-4) or equivalent.

4) Methanol, HPLC grade (Fisher cat. #A452-4) or equivalent.

5) Polypropylene glycol, M.W. 425 (Aldrich cat. #20,230-4).

6) Polypropylene glycol, M.W. 1000 (Aldrich cat. #20,232-0).

7) Polypropylene glycol, M.W. 2000 (Aldrich cat. #20,233-9).

8) PPG tuning solution (for mass calibration of the LC/MS system). Dissolve 0.0014 g PPG 425, 0.0100 g PPG 1000, 0.0400 g PPG 2000, and 0.0126 g of ammonium formate in 50 mL of methanol, 50 mL water, and 0.1 mL of acetonitrile. Mix well. Store refrigerated in an amber bottle. Dilute 1/50 with 1:1 MeOH:water before use.

9) Sample diluent: 5% acetonitrile/water + 0.1% acetic acid. Mix 50 mL of acetonitrile with 950 mL water and 1 mL of acetic acid.

10) Test analytes tuning solution, 0.05 ng/µL. Mix 1 mL of a 1 ng/µL mixed solution of analytes in acetonitrile with 19 mL of 50% acetonitrile/water with 0.1% acetic acid. Store at refrigerated or frozen temperature.

11) Water, HPLC grade (J.T. Baker cat. # 4218-03).

12) Mobile phase A: 0.1% acetic acid in water. Mix 1 mL of acetic acid with 999 mL of HPLC water.

14) Mobile phase B: Acetonitrile.

15) Mesotrione, AMBA, and MNBA, Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

### 2.3 Safety and Health

Whereas most of the chemicals used and analyzed in this method have not been completely characterized, general laboratory safety is advised (e.g., safety glasses, gloves, etc. should be used).

## 3.0 ANALYTICAL PROCEDURE

Note: All glassware should be thoroughly cleaned and followed with a rinse of acetonitrile or methanol prior to use. The analysis system is very sensitive and may detect contamination from previous samples if all glassware is not properly cleaned prior to each use.

(Note: Samples must be shaken or mixed well prior to analysis using suitable sample preparation techniques.)

1) Allow samples to warm to ambient temperature.

2) Transfer 5 mL of sample into a 15 mL centrifuge tube and add 1 drop of acetic acid into the sample.

3) Sample fortification, if required for this particular sample, is to be done at this time.

4) Transfer the sample into an autosampler vial and analyze by LC/MS reversed-phase HPLC as detailed in Table 1 for the presence of Mesotrione, AMBA, and MNBA.

## 4.0 INSTRUMENTATION

### 4.1 Description and Operating Conditions: HPLC

See Table 1 for a description of the HPLC system and chromatographic conditions. These tables also describe typical MS state file values and for conditions used with the Ion-Sprayinterface in the Analytical Method 6179-04. The optimized values for the analytes state files may vary with time and need periodic re-optimization by infusion of the analytes into the mass spectrometer.

## 4.2 Description and Operating Conditions: LC/MS

Mesotrione, AMBA, and MNBA are monitored as negative ions. Triple stage quadrupole analysis (MS/MS) of the unique precursor/product ion pair is suggested, although single stage quadrupole analysis (MS) utilizing the molecular ion may be performed provided that no interferences are present in the sample matrix. The optimized values for the Ion-Spray interface may vary with time and may need to be periodically re-optimized.

## 4.3 Calibration and Standardization: LC/MS

- Calibrate and tune the mass spectrometer prior to analyzing samples. Check the calibration and tune by infusing a standard solution of polypropylene glycol (PPG) into the mass spectrometer using the Ion-Sprayinterface while monitoring positive ions. Weekly calibrations and tunes with the PPG solution are considered sufficient provided that instrument mass calibration stability is demonstrated for that time interval.

- Determine the specific ion to monitor for each analyte by infusion of an analyte test solution (approx. 0.05-0.2 ng/µL in 50% acetonitrile/water, 0.1% acetic acid) while scanning the Q1 quadrupole mass analyzer to find the optimum ion. Determine the specific product ion fragment to monitor for each analyte in the MS/MS mode by passing the characteristic precursor ion through Q1, fragmenting the ion in Q2, and scanning the resulting ion fragments in Q3. The selected product ion chosen to monitor will depend on the intensity of the ion fragment along with the possibility that an interference also has the same fragment ion. Table 2 lists the precursor ion and monitored product ion for each analyte. Typical Ion-Spraymass fragmentation spectra are presented in Figure 3.

- Determine the retention time of the analytes by injecting a standard solution into the HPLC. During a series of analyses, the analyte retention time should vary no more than 2% from its mean value, on a daily basis.

- Calibrate the instrument by constructing a calibration curve from detector response (chromatographic peak height or area) and the amount of analyte injected, encompassing a range from 1.25 to 50 pg (50 µL injection). The response curve can be constructed manually or, preferably, by generation of a linear regression equation by use of a computer or appropriate calculator. Typical calibration data and chromatograms of calibration standards are presented in Figure 4.

## 5.0 INTERFERENCES

There are no known interferences originating from the sample. However, interferences can originate from impure chemicals, solvents, contaminated glassware, and the HPLC water supply.

## 6.0 CONFIRMATORY TECHNIQUES

No confirmatory analysis procedure is included in this method. This method employs highly specific LC/MS/MS for the detection mode, coupled with the characteristic retention time observed for the anlayte on the appropriate HPLC column.

## 7.0 TIME REQUIRED

For a set of 20 samples, sample preparation and data compilation can be completed in an eight-hour working day. The HPLC analysis requires approximately 15 minutes.

## 8.0 MODIFICATIONS AND POTENTIAL PROBLEMS

1) Contaminants from chemicals, solvents, glassware, and the HPLC water supply can interfere with the analysis. It is recommended that a reagent blank be run with an analysis set to verify that no interferences are originating from the chemicals and reagents used in this procedure. MS techniques are very sensitive. All glassware should be solvent rinsed before use to prevent inadvertent contamination of control or low level samples.

2) Analytical Method 6179-04 was validated only for the water type listed in the final method. Other water samples from different locations may exhibit interference problems which were not observed with these samples.

3) No analyte stability or solubility problems have been observed when solutions have been prepared and stored as detailed in Section 9.

4) Long-term optimization of the LC/MS signal by infusion of a test mixture of analytes into the system will result in lingering high backgrounds for the molecular ions. While the background signals will decrease with time or cleaning of the orifice plate, it may be severe enough to affect the ability to achieve desired signal to noise ratios for lowest standards. For this reason it is highly recommended that optimizing/calibrating with analytical standards be done with dilute solutions and the optimizing/calibrating time be minimized. It is also recommended after calibrating/optimizing with test analytes, to turn the power off to the electronics,

remove the Ion-Spray interface, and thoroughly wipe clean the orifice plate using a lint-free tissue wetted with methanol. Repeat several times.

5) This method has been tested only on a Micromass Quattro Ultima LC/MS system using the Ion-Spray interface. Different brand or model of LC/MS system with Ion-Spray interface may be used if the sensitivity of all analytes are acceptable.

6) Reversed-phase column from other manufacturers may be substituted for the column used in this study provided that the analyst demonstrates acceptable peak shape and sensitivity. Mesotrione may chelate with metal ion impurity present in the silica based HPLC column and cause peak tailing. High purity silica column or particularly polymer column is highly recommended. The recommended alternative column is Luna Phenyl-Hexyl 3 um 50x3 mm (Phenomenex Cat# 00B-4256-Y0). On this column AMBA may eluted before MNBA.

7) Inject several standard solution (at least five injections) to prime the column and establish the retention time before starting the analysis.

## 9.0 PREPARATION OF STANDARD SOLUTIONS

All individual standard solutions are stored in amber bottles in a refrigerator (< 5°C) when not in use. Mixed standards solutions are also stored in a refrigerator. The mixed standards are used for fortifications and HPLC standards.

Prepare individual 100 ng/µL stock solutions for Mesotrione, AMBA, and MNBA. Weigh approximately 10.0 mg of analyte. Determine the appropriate volume of solvent to add using the equation presented below. The concentration of the analytical standard is corrected for its chemical purity. Methanol is used as the solvent for Mesotrione, AMBA, and MNBA. The stock solution should be stored in a refrigerator up to 6 months.

$$V\,(mL) = \frac{W(mg) \times P}{C\,(ng/\mu L)} \times 10^3$$

Where V is the volume of solvent needed; W is the weight, in mg, of the solid analytical standard; P is the purity, in decimal form, of the analytical standard; C is the desired concentration of the final solution, in ng/µL; and $10^3$ is a conversion factor.

For example:

The volume required to dilute 9.9 mg of an analyte, of 98.0% purity, to a final concentration of 100 ng/µL is:

$$V\,(mL) = \frac{9.9\,mg \times 0.98}{100\,ng/\mu L} \times 10^3 = 97.02\,mL$$

Fortification and calibration standards are prepared from the analyte stock solutions. Prepare a 10 ng/µL mixed solution by pipetting 10.0 mL of each analyte 100 ng/µL stock solution into a 100-mL volumetric flask and then diluting to the calibration mark with sample diluent This standard mix solution should be stored in a refrigerator up to 6 months. Subsequent dilutions of this solution with 0.1% acetic acid solution will depend upon the desired fortification level(s). Fortification standards should be prepared such that no more than 1.0 mL of the fortification solution is added to a sample. (Example: For a 9.0 mL water sample, the addition of 1.0 mL of a 0.5 pg/µL fortification solution will result in a fortification level of 0.05 ppb.). This fortification standard should be store in a refrigerator for up to 1 week.

Prepare a 0.1 ng/µL mixed standard for generating external calibration curves for Mesotrione, AMBA, and MNBA on the LC/MS system. Pipette 1.0 mL from the 10 ng/µL mixed standard solution into a 100-mL volumetric flask and dilute to the calibration mark using 0.1% acetic acid solution. Subsequent dilutions using sample diluent are made to prepare a series of calibration standards. The calibration standard solution should be store in a refrigerator for up to 1 week.

# 10.0 METHODS OF CALCULATION

## 10.1 Determination of Residues in Samples

Inject the sample solution from Section 3.3 into the HPLC/MS system. The sample solution may be diluted if the analyte response exceeds the range of the calibration curve. The amount of analyte injected (pg) is determined by entering the value of the chromatographic peak height or area, in the calibration response curve (Section 9) and calculating (by computer, calculator, or manual means) the corresponding value of picograms injected. Typical chromatograms for fortified water are presented in Figures 6-8.

## 10.2 Determination of Residues in Fortified Samples

Validate the method for each set of samples analyzed by including a control sample and one or more control samples fortified prior to the extraction procedure with 0.05 ppb or more of each analyte in water.

Add an appropriate volume of a fortification solution (from Section 9) to the sample prior to injection. The total volume of the added fortification solution should not exceed 1.0 mL. Proceed with the sample injection.

## 10.3 Calculations

Calculations may be performed by computer program or manually as follows:

Calculate the analyte concentration (in ppb) for field samples from equation (1):

$$(1)\ \text{ppb analyte} = \frac{\text{pg analyte found}}{\text{mg sample injected}} \text{X} \frac{1}{\text{R}}$$

where R is the recovery factor expressed in decimal form (i.e., 0.8 = 80%) and is calculated from equation (2), and the chemical purity of the analytical standard has been accounted for in the preparation of the standard solutions. The use of the recovery correction factor "1/R" is left to the discretion of the study director. One gram of water is equal to 1 mL of water.

The recovery factor, expressed as a percentage (R%), is calculated from fortification experiments and is presented in equation (2).

$$(2)\ \text{R\%} = \frac{\text{ppb analyte found - ppb analyte (control)}}{\text{ppb analyte added}} \text{X}\, 100\%$$

The accuracy of the method is determined by the average recovery of the analytes fortified into the test substrate. The precision is estimated by the relative standard deviation of the determined concentration.

If background interference is found in the matrix blank (water control), it will be reported in the data table. In addition, an indication must be made as to whether or not these amounts were taken into account in the recovery calculations. The decision of whether to subtract any amounts found in the matrix blank from the recovery sample(s) is left to the discretion of the study director.

## 13.0 TABLES AND FIGURES

### TABLE 1. HPLC SYSTEM AND OPERATING CONDITIONS

**Instrumentation:**

Waters model 2690 HPLC system

**Operating Conditions**
Column Heater: 35 °C
Injector Volume: 50 µL
Mobile Phase Flow Rate: see below
Column: Phenomenex Polymerx RP-1 guard column (cat# AJO-5809, Phenomenex) equipped with a guard column holder (cat# KJO-4282, Phenomenex) PLRP-S 100 A°, 5µm 50x4.6 mm (cat# 1512-1500, Polymer Laboratories) An Upchurch (A-318) pre-column filter (0.5 µm) is also installed between the autosampler and columns to prevent fine particles from the sample blocking the columns.

Mobile phase A = 0.1% acetic acid in HPLC water
B = Acetonitrile

Mobile Phase Gradient Program:

| Run Time (min.) | % A | % B | Flow (mL/min) | Curve |
|---|---|---|---|---|
| 0 | 98 | 2 | 0.5 | 1 |
| 0.5 | 98 | 2 | 0.5 | 1 |
| 7.0 | 5 | 95 | 0.5 | 6 |
| 10 | 5 | 95 | 0.5 | 1 |
| 15 | 98 | 2 | 0.8 | 1 |

Total Run Time: 15 min
Analyte Retention Times:

| | | |
|---|---|---|
| MNBA | 4.35 | min |
| AMBA | 5.48 | min |
| Mesotrione | 7.24 | min |

Divert the column flow to waste before 3.5 min and after 9.0 min of the run time using a valco 6 port switching valve. This will keep the interface clean.

## TABLE 2. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS: LC/MS/MS

**Instrumentation:**

Micromass Quattro Ultima

**Software:**

Masslynx 4.0

**Operating Conditions:**

**Ion-Spray Interface**

Instrument Parameters

| | |
|---|---|
| Polarity | ES- |
| Calibration | Static 2 |
| Capillary (kV) | 0.75 |
| Cone (V) | vary |
| Hex 1 (V) | 0.0 |
| Aperture (V) | 0.0 |
| Hex 2 (V) | 0.0 |
| Source Temperature (°C) | 125 |
| Desolvation Temperature (°C) | 400 |
| Cone Gas Flow (L/Hr) | 94 |
| Desolvation Gas Flow (L/Hr) | 1008 |
| LM 1 Resolution | 12 |
| HM 1 Resolution | 12 |
| Ion Energy 1 | 0.5 |
| Entrance | -5 |
| Exit | 1 |
| LM 2 Resolution | 12.0 |
| HM 2 Resolution | 12.0 |
| Ion Energy 2 | 0.5 |
| Multiplier (V) | 653 |

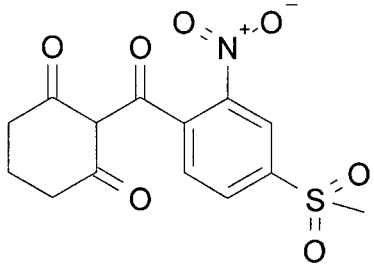
## TABLE 2. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS: LC/MS/MS (Continued)

**Tuning Method Report**

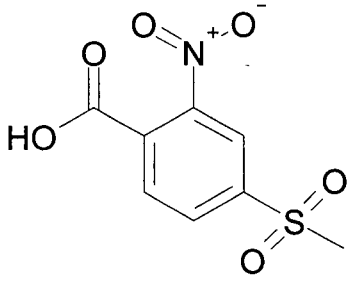
| | |
|---|---|
| Type | MRM |
| Ion Mode | ES- |
| Dwell time | 0.01 sec |
| Interchannel delay | 0.1 |
| Interscan delay | 0.1 |

| period | analyte | start (min) | End (min) | Dwell Time(s) | collision energy | cone voltage | molecular ion | product ion |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| 1 | MNBA | 3 | 5 | 1.0 | 10 | 40 | 244.0 | 199.8 |
| 2 | AMBA | 5 | 6.5 | 0.6 | 15 | 20 | 214.0 | 169.9 |
| 3 | Mesotrione | 6.5 | 9 | 2.5 | 10 | 40 | 338.2 | 291.0 |

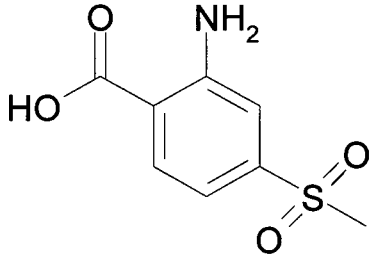
## FIGURE 1. CHEMICAL NAMES AND STRUCTURES



Mesotrione ( ZA 1296 )
CAS Number: 104206-82-8
2-(4-methylsulfonyl-2-nitriobenzoyl)-1,3-cyclohexanedione



AMBA (NOA-422848)
CAS Number: 393085-45-5
Benzoic acid, 2-amino-4-(methylsulfonyl)-



MNBA (NOA-437130)
CAS Number: 110964-79-9
Benzoic acid, 4-(methylsulfonyl)-2-nitro-

## FIGURE 2. METHOD 6179-04 FLOW DIAGRAM FOR WATER

Acidify sample with acetic acid

↓

Transfer to micro vial

↓

Analyze sample by LC/MS