ATTACHMENT I – Residue Method RM-35WM

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DETERMINATION OF MeBA and DesMe-2023 IN WATER
METHOD RM-35-WM

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INTRODUCTION

This method determines residues of two metabolites of V-10029 (KIH-2023): MeBA and DesMe-2023 in water. This method is derived from methods developed by ABC Laboratories, Inc. for Kumiai Chemical Industry Co., Ltd[1,2].

MeBA and DesMe-2023 residues are separately analyzed by high performance liquid chromatography (HPLC) using a variable wavelength UV detector set at 250 nm. Prior to analysis, the MeBA and DesMe-2023 are separated by passing the sample through a C_{18} solid phase extraction (SPE) column. The MeBA is eluted from the SPE column using water and the DesMe-2023 is eluted using methanol:water (1:1, v/v).

REAGENTS

Acetic acid - glacial, reagent grade or equivalent.

Methanol - pesticide quality or equivalent.

Phosphoric acid - concentrated, reagent grade or equivalent.

Potassium phosphate - monobasic (KH_2PO_4), reagent grade or equivalent.

Sodium phosphate - dibasic, heptahydrate ($Na_2HPO_4 \cdot 7H_20$), reagent grade or equivalent.

Water-deionized.

Water- HPLC grade or equivalent.

REAGENT SOLUTIONS

Acetic acid - 0.05 *N* - Add 3 mL of glacial acetic acid to a 1L volumetric flask, partially filled with deionized water. Dilute to volume with deionized water, then stopper and shake. Store at room temperature.

Methanol:water - 1:9, v/v - Combine 1 part of methanol with 9 parts of deionized water. For example, add 100 mL of methanol to 900 mL of deionized water. Mix well and store at room temperature.

Methanol:water - 1:1, v/v - Combine equal volumes of methanol and deionized water. For example, add 500 mL of methanol to 500 mL of deionized water. Mix well and store at room temperature.

Phosphoric acid - 0.05% v/v - Add 0.25 mL of concentrated (85%) phosphoric acid to 500 mL of HPLC water. Mix well and store at room temperature.

Potassium phosphate - 0.2 *M* - Dissolve 5.44 grams of KH_2PO_4 in 200 mL of deionized water. Mix well and store at room temperature. (Amounts used may be scaled, as appropriate).

Sodium phosphate - 0.2 *M* - Dissolve 5.36 grams of $Na_2HPO_4 \cdot 7H_20$ in 100 mL of deionized water. Mix well and store at room temperature. (Amounts used may be scaled, as appropriate).

Phosphate buffer - 0.2 *M* - pH 6 - Add 0.2 *M* Na_2HPO_4 to KH_2PO_4 to adjust the pH to 6. Store at room temperature.

Phosphate buffer - 0.01 *M* - pH 6 - Combine 1 part of 0.2 *M* phosphate buffer with 19 parts of deionized water. For example, combine 25 mL of 0.2 *M* phosphate buffer with 475 mL of deionized water. Store at room temperature.

REFERENCE STANDARDS

MeBA - analytical standard of known purity. Prepare a stock solution containing 1.0 mg/mL in methanol:water (1:1, v/v). Prepare fortifying solutions containing 1.0 and 0.10 µg/mL by diluting the stock solution with HPLC grade water. Prepare a minimum of four linearity standards by diluting the stock solution with deionized water to concentrations ranging from 0.01 to 0.2 µg/mL (See Note 1). Prepare a calibrating solution containing 0.10 µg/mL by diluting the stock solution with water. (The calibrating solution may be used as one of the four required linearity standards). All solutions should be kept refrigerated when not in use.

REFERENCE STANDARDS (CONTINUED)

DesMe-2023 - analytical standard of known purity. Prepare a stock solution containing 1.0 mg/mL in HPLC grade water. Prepare fortifying solutions containing 1.0 and 0.10 µg/mL by diluting the stock solution with HPLC grade water. Prepare a minimum of four linearity standards by diluting the stock solution with 0.01 *M* phosphate buffer to concentrations ranging from 0.01 to 0.2 µg/mL (See Note 1). Prepare a calibrating solution containing 0.10 µg/mL by diluting the stock solution with 0.01 *M* phosphate buffer. (The calibrating solution may be used as one of the four required linearity standards). All solutions should be kept refrigerated when not in use.

EQUIPMENT

Baker SPE-12G Column Processor (12-port vacuum manifold) - J.T. Baker Product # 7018-00 or equivalent system.

Bakerbond C_{18} Disposable Extraction Columns - 6 mL, Cat # 7020-6. See Note 2.

Beakers - 100 mL.

Filter funnels - glass, 10 cm diameter.

Glass wool.

Liquid Chromatograph - Hewlett-Packard Model 1050, equipped with a tertiary pump, an autosampler, an HP ChemStation, and an HP Model 79853A Variable Wavelength UV detector or equivalent system.

Pasteur pipets - 5¾" and 9".

Rotary evaporator - Büchi or equivalent, equipped with a temperature controlled water bath.

Round-bottom flasks - 50 mL, 250 mL.

Syringe - Tuberculin, with glass Luer-Tip, 1 mL.

Syringe filters - Gelman Acrodisc LC13 (PVDF, 0.45 µm).

Ultrasonic cleaner - Branson 3200 or equivalent.

Vials - glass, 6 and 11 dram capacity, with polyethylene lined screw caps or equivalent.

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ANALYTICAL PROCEDURES

1. SPE Cleanup (See Note 2)

Attach a C_{18} disposable extraction column to the Baker-SPE vacuum manifold. Pre-condition the column with 30 mL deionized water, followed by 10 mL of methanol, then 30 mL of deionized water. **Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied.**

Filter a portion of the sample through a filter funnel and a small plug of glass wool to remove any foreign material and/or sediment present. Weigh 20 grams of the filtered sample into a 100 mL beaker (or similar sized container). At this point, if required by the testing facility, control samples to be used for method recoveries may be fortified with MeBA and DesMe-2023 (See Note 3). Place an 11 dram vial (or similar sized container) under the column to collect the MeBA eluate. Transfer the sample to the column and apply gentle vacuum to achieve a flow rate of approximately 1 drop/second. Release the vacuum when the sample reaches the top of the column packing. **Do not allow the column to dry.**

Add 10 mL of deionized water to the beaker that the sample was weighed in, swirl and sonicate, then transfer to the column. Apply gentle vacuum to the column until this rinsate reaches the top of the column packing. Add 10 mL of deionized water to the column and elute under vacuum until all the water has been drawn through the column. Remove the vial containing this eluant (MeBA fraction) and reserve for **Step 2, Analysis for MeBA.**

Re-apply vacuum to the column for approximately 2-3 minutes to thoroughly dry the column. Place a 6 dram vial (or similar sized container) under the column and add 5 mL of methanol:water (1:9, v/v) to the column. Apply gentle vacuum until all the methanol:water has been eluted. Discard this eluate. Place a clean, dry, 6 dram vial (or similar sized container) under the column and add 5 mL of methanol:water (1:1, v/v) to the column. Apply gentle vacuum until all the methanol:water has been eluted. Remove this vial (DesMe-2023 fraction) and reserve for **Step 3, Analysis for DesMe-2023.**

2. Analysis for MeBA

Transfer the MeBA eluate from step 1 to a 250 mL round-bottom flask using three 1 mL portions of deionized water to rinse the vial. Add 1 mL of 0.05 *N* acetic acid solution to the flask (to minimize evaporative losses of MeBA), then evaporate to approximately 5-10 mL using a rotary-evaporator and water bath set to 35-45°C (a minimum vacuum of 29 inches of Hg required). Transfer the extract to a 50 mL round-bottom flask, using three 1 mL portions of deionized water to rinse the 250 mL round-bottom flask, then evaporate the extract to dryness using a rotary-evaporator and water bath set to 35-45°C.

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Re-dissolve the extract in 1.0 mL of deionized water and sonicate for approximately 15 seconds. Attach an Acrodisc filter to a 1 mL glass syringe (plunger removed) and transfer the sample extract to the syringe with a Pasteur pipet. Insert the syringe plunger into the syringe and gently push the extract through the filter into an autosampler vial. Seal and store at ≤0°C until HPLC analysis.

Analyze the sample extracts, along with calibrating standard solutions, using the following (or similar) operating conditions:

Column: Phenomenex Prodigy ODS (3) (250 mm x 4.6 mm, 5 µm particle size), Phenomenex Cat # 00G-4097-E0 or equivalent.
Column Temperature: 35°C
Mobile phase: 7.0% A and 93.0% B, where A = methanol and B = 0.05% H_3PO_4.
Run Time: 10 minutes + 10 minute post time (to allow for elution of matrix coextractives)
Flow rate: 1.0 mL/min.
Injection volume: 50 µL
Detector Wavelength: 250 nm
MeBA Retention Time: 6.4 minutes.

The HPLC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labeled with the parameters used.

The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. The calibrating standard vials contain 0.10 µg/mL of MeBA in water. This sequence may, however, be modified if the reproducibility requirement is met. See Note 4. Each sequence must begin and end with a calibration standard.

3. Analysis for DesMe-2023

Transfer the DesMe-2023 eluate from step 2 to a 50 mL round-bottom flask using three 1 mL portions of methanol:water (1:1, v/v) to rinse the vial, then evaporate to dryness using a rotary-evaporator and water bath set to 35-45°C (a minimum vacuum of 29 inches of Hg required). Re-dissolve the extract in 1.0 mL of 0.01 *M* phosphate buffer and sonicate for approximately 15 seconds. Attach an Acrodisc filter to a 1 mL glass syringe (plunger removed) and transfer the sample extract to the syringe with a Pasteur pipet. Insert the syringe plunger into the syringe and gently push the extract through the filter into an autosampler vial. Seal and store at ≤0°C until HPLC analysis.

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Analyze the sample extracts, along with calibrating standard solutions, using the following (or similar) operating conditions:

Column: Phenomenex Prodigy ODS (3) (250 mm x 4.6 mm, 5 µm particle size), Phenomenex Cat # 00G-4097-E0 or equivalent.

Column Temperature: 35°C
Mobile phase: A = methanol; B = water + 0.05% H_3PO_4 (v/v).
Gradient:
Time 0, 40% A + 60% B
Time 3 minutes, 40% A + 60% B
Time 13 minutes, 80% A + 20% B
Time 20 minutes, 80% A + 20% B

Run Time: 20 minutes + 5 minute post time (to equilibrate to initial mobile phase)
Flow rate: 1.0 mL/min.
Injection volume: 50 µL
Detector Wavelength: 250 nm
DesMe-2023 Retention Time: 13.1 minutes

The HPLC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences (see discussion of interferences below). Each set of chromatograms must be clearly labeled with the parameters used.

The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. The calibrating standard vials contain 0.10 µg/mL of DesMe-2023 in water. This sequence may, however, be modified if the reproducibility requirement is met. See Note 4. Each sequence must begin and end with a calibration standard.

CALCULATIONS

The concentration of MeBA and DesMe-2023 in each sample is calculated using the following formula:

$$ppm\ MeBA / DesMe-2023 = \frac{B \times C \times V \times DF}{A \times W}$$

where:

B = integration counts for MeBA/DesMe-2023 in the sample.

C = concentration of MeBA/DesMe-2023 in calibrating standard (0.1 µg/mL).

V = final volume of the sample extract (1.0 mL).

DF = dilution factor, used if the sample extract is diluted prior to analysis.

A = mean integration counts for MeBA/DesMe-2023 in the calibrating standards.

W = sample weight (20 grams).

INTERFERENCES

V-10029 can potentially interfere with the analysis of DesMe-2023 because the parent compound and its metabolite coelute from the C_{18} disposable extraction column used in this method. V-10029 and DesMe-2023 are well separated by HPLC, using the conditions described above (V-10029 elutes approximately 3 minutes after the DesMe-2023). If any changes to the HPLC conditions are made, however, the retention time of V-10029 should be confirmed to ensure that an interference will not occur.

LIMITS OF DETECTION AND QUANTITATION

The validated limit of detection (LOD) of MeBA/DesMe-2023 in water analyzed by this method is 0.0005 ppm (0.5 ppb). The validated limit of quantitation (LOQ) is 1.0 ppb.

ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of eight samples for MeBA/DesMe-2023 in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. At Valent, linearity of the HPLC must be determined each day that samples are analyzed (Valent SOP #VR-007). Linearity is determined by analyzing a series of linearity standards containing 0.01 to 0.20 µg/mL of MeBA/DesMe-2023. The response for each standard is normalized to response per 1.0 µg/mL by dividing the response of each standard by its concentration. The coefficient of variation (CV) of these responses must be 10% or less. Sample extracts must be diluted to bring the concentration of MeBA/DesMe-2023 within the range of linearity established.

2. Each lot of C_{18} disposable extraction columns must be checked for recovery of MeBA/DesMe-2023 as follows: Transfer 1.0 mL of the 0.10 µg/mL MeBA calibrating standard solution and 1.0 mL of the 0.10 µg/mL DesMe-2023 calibrating standard solution to a 50 mL round-bottom flask and evaporate to dryness using a rotary-evaporator with a water bath temperature set to 35-45°C. Re-dissolve in 20 mL of water and transfer to a C_{18} disposable extraction column. Elute the MeBA/DesMe-2023 as described under **Step 1, SPE Cleanup.**

   Analyze the MeBA eluate as described under **Step 2, Analysis for MeBA** and the DesMe-2023 as described under **Step 3, Analysis for DesMe-2023**. If either the MeBA or DesMe-2023 peak for the eluate is less than 90% of the calibrating standard, then the elution profile for both analytes must be determined.

3. At Valent, untreated control (UTC) samples are always fortified and analyzed with each set of study samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

   The level of fortification is generally 1 ppb (the LOQ of the method) and/or 5 ppb of each analyte. The 1 ppb fortifications are made by adding 200 µL of each 0.10 µg/mL fortifying solution to a 20 gram sample. The 5 ppb fortifications are made by adding 100 µL of each 1.0 µg/mL fortifying solution to a 20 gram sample. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

4. At Valent, reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, these CV's must be 10% or less unless approved by the chemist responsible for the analysis (Valent SOP #VR-013).

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REFERENCES

1. Kaiser, F. E., *Liquid Chromatographic Method for the Determination of DesMe-2023 in Water*, ABC Laboratories, Inc.

2. Kaiser, F. E., *Liquid Chromatographic Method for the Determination of Me_2BA in Arkansas Water*, ABC Laboratories, Inc.

METHOD APPROVAL

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