
ABSTRACT

This report presents the analytical results from a study to determine the relative magnitude of combined bifenazate and UCC-D3598 residues in mint tops and mint oil following exaggerated-rate treatment of mint with UCC-D2341 50WP. The mint tops and mint oil samples were received by NCL from the processing facility, USDA/ARS Irrigated Agriculture Research and Extension Center of Prosser, WA.

The analytical method for the determination of combined bifenazate and UCC-D3598 residues in mint tops began with the extraction of the sample with 0.1% acetic acid in acetonitrile. A portion of the extract was then diluted in an ascorbic acid reducing solution, incubated, filtered, and analyzed using a liquid chromatograph with a tandem mass spectrometer (LC/MS/MS). In the analytical method for the determination of bifenazate and UCC-D3598 residues in mint oil, a measured amount of mint oil was diluted appropriately with an ascorbic acid reducing solution, incubated, and analyzed by LC/MS/MS. In both methods, the bifenazate metabolite, UCC-D3598, was reduced to bifenazate for quantitation. Therefore, the analytical residue result from an experimental sample represents the combined total of bifenazate and UCC-D3598 residues in the sample.

The target limits of quantitation (LOQ) were 0.010 $\mu\text{g/g}$ (ppm) for both bifenazate and UCC-D3598 in/on mint tops and mint oil. To verify method performance from the LOQ to the highest anticipated residues, method validation sets were performed for each analyte in/on each matrix using untreated control samples. The methods were validated for the respective matrix and for each analyte at a minimum of three fortification levels interspersed between the 0.010 $\mu\text{g/g}$ and 50 $\mu\text{g/g}$. Each validation set also included two unfortified control samples and a reagent blank. The method validation fortification levels for mint tops were 0.010 $\mu\text{g/g}$ (1X LOQ), 1.0 $\mu\text{g/g}$ (100X LOQ), and 50 $\mu\text{g/g}$ (5,000X LOQ). The mint oil method was validated twice, once for an LOQ of 0.050 $\mu\text{g/g}$, and then, after these results indicated that a lower LOQ was possible, for an LOQ of 0.010 $\mu\text{g/g}$. The fortification levels for the first mint oil validation set were 0.050 $\mu\text{g/g}$ (5X LOQ), 1.0 $\mu\text{g/g}$ (100X LOQ), and 50 $\mu\text{g/g}$ (5,000X LOQ), and the fortification levels for the second validation set were 0.010 $\mu\text{g/g}$ (1X LOQ), 0.20 $\mu\text{g/g}$ (20X LOQ), and 10 $\mu\text{g/g}$ (1,000X LOQ).

In the validation set for bifenazate in/on mint tops, and over three fortification levels, recoveries ranged from 75.8% to 107% and the mean percent recovery (mean $\pm \sigma_{n-1}$) was $94.3 \pm 8.6\%$. In the validation set for UCC-D3598 in/on mint tops, and over three fortification levels, recoveries ranged from 92.1% to 114% and the mean percent recovery (mean $\pm \sigma_{n-1}$) was $100 \pm 9.0\%$. Standard deviations for the mean percent recoveries were calculated and designated as σ_{n-1} , and expressed as an absolute percent value.

In the validation sets for bifenazate and UCC-D3598 in/on mint oil, and over all six fortification levels, bifenazate recoveries ranged from 92.0% to 112% and the overall mean percent recovery (mean $\pm \sigma_{n-1}$) was $101 \pm 4.5\%$; UCC-D3598 recoveries ranged from 80.2% to 106% and the mean percent recovery (mean $\pm \sigma_{n-1}$) was $96.2 \pm 7.8\%$.

Residues for each experimental sample are reported uncorrected. The total bifenazate and UCC-D3598 residues in the three treated mint tops samples were 21 $\mu\text{g/g}$ (ppm), 21 $\mu\text{g/g}$ (ppm), and 19 $\mu\text{g/g}$ (ppm) and the total bifenazate and UCC-D3598 residues in the three treated mint oil samples were 1.6 $\mu\text{g/g}$ (ppm), 0.90 $\mu\text{g/g}$ (ppm), and 0.97 $\mu\text{g/g}$ (ppm). No quantifiable residues were detected in experimental control samples.

Single analyte method fortification samples for bifenazate and UCC-D3598, at 0.050 $\mu\text{g/g}$ (5X LOQ) and 1.0 $\mu\text{g/g}$ (100X LOQ), were extracted and analyzed with experimental sample(s). One analytical set was extracted for the mint tops samples; recoveries from bifenazate fortifications in/on mint tops were 87.6% and 97.5% and recoveries from UCC-D3598 fortifications in/on mint tops were 89.8% and 95.7%. Three analytical sets were extracted for the mint oil samples; recoveries from bifenazate fortifications in/on mint oil ranged from 98.2% to 121% and recoveries from UCC-D3598 fortifications in/on mint oil ranged from 90.0% to 120%.

INTRODUCTION

The following report presents the analytical results from a study to determine the relative magnitude of combined bifentazate and UCC-D3598 residues in mint tops and mint oil following exaggerated-rate treatment of mint with UCC-D2341 50WP. Mint tops and mint oil sample extracts were analyzed using bifentazate and UCC-D3598 as the analytical reference standards. UCC-D3598 residues were reduced to bifentazate for quantitation and the extracts analyzed for bifentazate using a LC/MS/MS. The analytical residue result from an experimental sample represents the combined total of bifentazate and UCC-D3598 residues in the sample.

In addition to the analytical results, the information which follows summarizes the experimental design of the analyses in terms of the analytical reference standards, the test system, the analytical methodology, limits of quantitation, quality control design, and calculations. Copies of sample chromatograms from the analyses are presented in Appendix A; copies of residue data sheets can be found in Appendix B; and copies of the methods used can be found in Appendix C.

EXPERIMENTAL DESIGN

ANALYTICAL REFERENCE STANDARDS

The analytical reference standards were supplied by Uniroyal Chemical Company, World Headquarters, Middlebury, CT.

<u>Name/experimental designation</u>	<u>Lot number</u>	<u>Percent purity</u>	<u>Expiration date</u>
Bifentazate	2614-86-SLD	98.1%	04/1/2004
UCC-D3598	2522-37A-HMB	99.95%	4/2002

Bifentazate = 2-(4-methoxy[1,1'-biphenyl]-3-yl)hydrazinecarboxylic acid, 1-methylethyl ester, CAS No. 149877-41-8.

UCC-D3598 = diazenecarboxylic acid, 2-(4-methoxy-[1,1'-biphenyl]-3-yl)-, 1-methylethyl ester, CAS No. 149878-40-0.

The analytical reference standards and standard solutions were stored at $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$.

TEST SYSTEM

The test system in this study consisted of mint tops and mint oil samples from one field trial in Yakima County, Washington (Region 11).^① The samples were processed at USDA/ARS Irrigated Agriculture Research and Extension Center, Prosser, Washington. After processing, the samples were shipped overnight via FedEx, on dry ice, to the analytical laboratory where they were stored at $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$.

LIMITS OF QUANTITATION

The limits of quantitation (LOQ) for each analyte, bifentazate and UCC-D3598, were the lowest validated levels of $0.010 \mu\text{g/g}$ (ppm) in/on mint tops and mint oil matrices.

ANALYTICAL METHODOLOGIES

The analytical methods used in this study were based on the principles outlined in *Method Used for the Analysis of Bifenazate and UCC-D3598 Residues in Cottonseed, Gin Trash, Meal, Hulls, and Refined Oil*, Report 10495-1, Ricerca, Inc., Painesville, OH. Complete copies of the analytical methods used are presented in Appendix C. NCL ME 228 was used for mint oil sample analyses and NCL ME 229 was used for mint tops sample analyses^②

Preparation of standard solutions for mint tops and mint oil analyses

For the calculations involved in the preparation of the following standard solutions, see the calculations section of this report.

- Preparation of a $1,000 \text{ ng}/\mu\text{L}$ bifentazate stock standard solution and a $1,000 \text{ ng}/\mu\text{L}$ UCC-D3598 stock standard solution:

An aliquot, 8-10 mg, of the respective neat analytical standard was weighed out and, taking into account the percent purity, was brought to volume with the appropriate amount of acetonitrile to yield a $1,000 \text{ ng}/\mu\text{L}$ standard solution.

- Preparation of $100 \mu\text{g}/\text{mL}$, $10 \mu\text{g}/\text{mL}$, $2.0 \mu\text{g}/\text{mL}$, $1.0 \mu\text{g}/\text{mL}$, $0.10 \mu\text{g}/\text{mL}$ and $0.020 \mu\text{g}/\text{mL}$ (ppm) stock standard solutions for bifentazate or UCC-D3598

Stock standard solutions at $100 \mu\text{g}/\text{mL}$, $10 \mu\text{g}/\text{mL}$, $2.0 \mu\text{g}/\text{mL}$, $1.0 \mu\text{g}/\text{mL}$, $0.10 \mu\text{g}/\text{mL}$ and $0.020 \mu\text{g}/\text{mL}$ (ppm) for bifentazate or UCC-D3598 were prepared by serial dilution in acetonitrile from the $1,000 \mu\text{g}/\text{mL}$ (ppm) stock standard solution of the respective analyte.

^①Mint oil samples for fortification within this study were obtained from another source, and were not generated within this study or under GLPS.
^②Although ME228 was validated for mint oil, data generation & processing parameters from ME229 were used to analyze experimental mint oil samples.

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- Preparation of bifenazate working standard instrument calibration solutions

Bifenazate working standard instrument calibration solutions were prepared for each analysis at six concentrations, 0.5X, 1X, 2X, 4X, 10X and 20X LOQ. The standards were prepared by diluting aliquots of stock standard solutions in dilution solution (20 mL 0.25% acetic acid in ACN + 20 mL 0.25 % ascorbic acid in 5:95 acetonitrile: water).

Example preparations:

Mint tops analyses: A 1X LOQ working standard instrument calibration solution was prepared by bringing 12.5 μL of a 0.020 ng/ μL bifenazate standard solution to 1.0 mL volume in dilution solution.

Mint oil analyses: A 1X LOQ working standard instrument calibration solution was prepared by bringing 5 μL of a 0.020 ng/ μL bifenazate standard solution to 1.0 mL volume in dilution solution.

Other concentrations of working standards were prepared in a similar manner using the 0.020 $\mu\text{g}/\text{mL}$ or the 0.10 $\mu\text{g}/\text{mL}$ bifenazate stock standard solutions.

All stock and working standard solutions were stored at $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$.

Summary of mint tops analytical method (NCL ME 229)

Homogenization:

The mint tops samples were homogenized using a Hobart Model 84145 Food Cutter and dry ice.

Fortification and extraction:

A 5.0 g aliquot of the mint tops sample was weighed into a Mason jar and, as required, untreated control samples were fortified. Single analyte fortifications were prepared by adding, using a microliter syringe, an appropriate aliquot (see calculations section) of the 1.0 ng/ μL , 100 ng/ μL or 1,000 ng/ μL ($\mu\text{g}/\text{mL}$) bifenazate or UCC-D3598 stock standard solutions.

Extraction solvent (1 mL glacial acetic acid brought up to 1L with ACN), 85 mL, was added to the Mason jar and the contents mixed using an Omni Mixer. After allowing the sample to remain in contact with the extraction solvent for ~5 minutes, the contents of the jar were swirled and then decanted into a 250 mL graduated mixing cylinder. The solids remaining in the extraction jar were rinsed twice with 5-10 mL aliquots of extraction solvent and the rinses added to the extract in the graduated cylinder. The volume of the extract was brought to 100 mL with extraction solvent and the contents of the cylinder were mixed. Approximately 40 mL of the extract were transferred to a 50 mL Teflon[®] centrifuge tube and centrifuged at ~10-12,000 rpm for ~10 minutes.

Reduction reaction (of UCC-D3598 residues to bifenazate):

A 2.5 mL aliquot of the supernatant was transferred to an 8 mL vial and then 2.5 mL of freshly prepared 0.25% ascorbic acid in 5:95 ACN:water solution and 37.5 μ L of a 10% acetic acid solution in water were added. The vial was capped, the contents shaken briefly to mix, and then the vial was placed in a 40-45°C water bath. After ~2 hours incubation, ~1 mL of the extract was transferred through a syringe tip filter (Alltech #6274, 13 mm, 45 μ , PP) into an autosampler vial for analysis.

Analysis:

Analysis was performed using a Phenomenex Luna, 2 mm x 100 mm, 3 μ , C18(2) column and a Perkin Elmer (PE) SCIEX API 3000 liquid chromatograph mass spectrometer mass spectrometer (LC/MS/MS) system, operated in multiple reaction mode and fitted with a Turbo Ion Spray unit operated in positive ion mode. Two Shimadzu LC-10 AD pumps were used with a SCL-10A Shimadzu controller and a PE Series 200 autosampler. Solvent A was HPLC grade water + 0.05% formic acid, and solvent B was HPLC grade ACN + 0.05% formic acid. The injection volume was 10 μ L (10 μ L loop being flushed with 50 μ L). The primary transition was 301-198 m/z and the confirmatory transition was 301-170 m/z.

Calibration procedure:

The calibration procedure consisted of injecting six working standard instrument calibration solutions throughout each run and calibrating using a linear regression curve. Standard concentrations were at are 0.5X, 1X, 2X, 4X, 10X, and 20X LOQ. The correlation coefficients (r-squared) of the standard regression curves were required to be at least 0.995. The regressions were calculated by the PE SCIEX Analyst Data System software. The software also calculated the sample residues printed on the data printouts, and these were then transcribed to the residue data sheets.

Summary of mint oil analytical method (NCL ME 228)

Fortification:

A 50 mg aliquot of the mint oil sample (measured as 54.5 μ L, see calculation section) was placed in an 8 mL glass tall-form vial. Untreated control samples were fortified as required, and then vortexed to mix. Single analyte fortifications were prepared by adding, using a microliter syringe, an appropriate aliquot (see calculations section) of the 0.10 ng/ μ L, 2.0 ng/ μ L, or 100 ng/ μ L (μ g/mL) bifenazate or UCC-D3598 stock standard solutions. A 1X LOQ fortification was prepared by adding 5 μ L of the 0.10 ng/ μ L stock standard to the 50 mg sample.

Reduction reaction (of UCC-D3598 residues to bifenazate):

A 2.5 mL aliquot of 0.25% acetic acid in ACN was added to the sample aliquot followed by 2.5 mL of 0.25% ascorbic acid in 5:95 ACN:water. The vial was capped and the contents shaken vigorously for ~2 minutes to dissolve the oil in the reducing solution. The vial was then placed in a 40-45°C water bath and, after ~2 hours incubation, ~1 mL of the extract was transferred to an autosampler vial for analysis.

Analysis:

As described for the analysis of mint tops.

Calibration procedure:

As described for the analysis of mint tops except that one of the five reported analyses was calibrated using a quadratic regression curve (the four other analyses were calibrated using a linear regression curve).

METHOD PERFORMANCE VALIDATIONS

To verify method performance from the LOQ to the highest anticipated residues, method validation sets were performed for each analyte in/on each matrix using untreated control samples. The methods were validated for the respective matrix and for each analyte at a minimum of three fortification levels interspersed between the 0.010 $\mu\text{g/g}$ and 50 $\mu\text{g/g}$. Each validation set also included two unfortified control samples and a reagent blank. The method validation fortification levels for mint tops were 0.010 $\mu\text{g/g}$ (1X LOQ), 1.0 $\mu\text{g/g}$ (100X LOQ), and 50 $\mu\text{g/g}$ (5,000X LOQ). The mint oil method was validated twice, once for an LOQ of 0.050 $\mu\text{g/g}$, and then, after these results indicated that a lower LOQ was possible, for an LOQ of 0.010 $\mu\text{g/g}$. The fortification levels for the first mint oil validation set were 0.050 $\mu\text{g/g}$ (5X LOQ), 1.0 $\mu\text{g/g}$ (100X LOQ), and 50 $\mu\text{g/g}$ (5,000X LOQ), and the fortification levels for the second validation set were 0.010 $\mu\text{g/g}$ (1X LOQ), 0.20 $\mu\text{g/g}$ (20X LOQ), and 10 $\mu\text{g/g}$ (1,000X LOQ).

SAMPLE ANALYSES AND QUALITY CONTROL

The mint tops and mint oil experimental sample extracts were analyzed for bifentazate residues. The analytical residue result from an experimental sample represented the combined total of bifentazate and UCC-D3598 residues in the sample. Residue results are reported uncorrected for method recovery, or for any trace residues. Analytical sample sets consisted of a reagent blank, one control sample, two single-analyte method fortified control samples (at 0.050 $\mu\text{g/g}$ and 1.0 $\mu\text{g/g}$) for each analyte, experimental treated samples, and calibration standards.

All fortified samples yielded acceptable method fortification recoveries between 70% and 120%, with two exceptions. In the mint oil sample sets extracted on 10/22/01 and 10/23/01, the 1.0 $\mu\text{g/g}$ bifentazate method fortification recoveries were both 121%. The Study Director was contacted and the data was accepted. The correlation coefficients (**r-squared**) of the bifentazate standard regression curves were > 0.995 .

CALCULATIONS

Threshold Area Counts

The term "NAC" (no area counts) found on the residue data sheet means that there were no area counts available to report at or above the area reject threshold. The area reject threshold was determined by the analyst to be at a level lower than 70% of the peak of the lowest calibration standard (0.5X LOQ) and which would exclude baseline noise.

Calculations for methods

- Calculation of the volume of mint oil equivalent to a weight of 50 mg

10 mL of mint oil (NCL sample #0109432-01A) was measured into a tarred 10 mL volumetric flask and weighed. The recorded weight was 9.18 g. Therefore the density of the mint oil was 0.918 g/mL (mg/ μ L).

If the density is 0.918 mg/ μ L, then 50 mg of oil is equivalent to:

$$\begin{aligned}(50 \text{ mg}) / (0.918 \text{ mg}/\mu\text{L}) &= 54.4662 \mu\text{L} \\ &= 54.5 \mu\text{L}\end{aligned}$$

- Calculation of the amount of solvent needed to bring a weighed amount of standard to the required concentration

$$\text{Volume of solvent} = \frac{\text{weight of standard (adjusted for percent purity)}}{\text{desired concentration}}$$

Example calculation for the preparation of the 1,000 ng/ μ L bifentazate stock standard solution:

$$\begin{aligned}\text{Weight bifentazate} &= 0.0084 \text{ g} \\ \text{Percent purity} &= 98.1\% \\ \text{Weight bifentazate corrected for percent purity} &= (0.0084 \text{ g}) (0.981) \\ &= 0.00824 \text{ g} \\ &= 8,240,000 \text{ ng}\end{aligned}$$

$$\begin{aligned}\text{Volume of solvent needed} &= \frac{8,240,000 \text{ ng}}{1,000 \text{ ng}/\mu\text{L}} \\ &= 8,240 \mu\text{L} \\ &= 8.24 \text{ mL}\end{aligned}$$

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- Calculations of the method dilution factors in the mint tops analytical method

The initial extract was brought to 100 mL.
2.5 mL of the 100 mL extract was taken through the reduction step.
This resulted in a dilution of 40 (100 mL / 2.5 mL).

This method dilution factor was incorporated into the preparation of the working standard instrument calibration solutions used in the mint tops sample analyses.

There was no method dilution factor in the mint oil analytical method.

NOTE: In both the mint tops and mint oil methods, the final extract volumes were 5.0 mL; this was incorporated into the preparation of the working standard instrument calibration solutions.

- Calculation of the amount of bifentazate or UCC-D3598 residues to be determined in a sample

Example calculation for a sample containing residues at 1X LOQ:

For a sample weight of 5.0 g, and with the established LOQ of 0.010 $\mu\text{g/g}$, the total amount of analyte to be detected at the LOQ was:

$$\begin{aligned}(5.0 \text{ g}) (0.010 \mu\text{g/g}) &= 0.050 \mu\text{g} \\ &= 50 \text{ ng analyte}\end{aligned}$$

- Calculation in the preparation of a bifentazate or UCC-D3598 method fortification

Example calculation for a 1X LOQ fortification:

A 1X LOQ, 0.010 $\mu\text{g/g}$ (ppm), fortification of analyte was prepared by fortifying a 5.0 g sample with 50 μL of a 1.0 $\text{ng}/\mu\text{L}$ stock standard solution.

$$\begin{aligned}(50 \mu\text{L}) (1.0 \text{ ng}/\mu\text{L}) / (5.0 \text{ g}) &= 10 \text{ ng/g} \\ &= 0.010 \mu\text{g/g}\end{aligned}$$

The amounts of standard solutions needed to prepare method fortifications at other concentrations were calculated in a similar manner.

- Calculations used in the preparation of bifenazate working standard instrument calibration solutions

Example calculations for a 1X LOQ standard for a mint tops analysis:

Taking into account that the total amount of analyte in an extract from a 1X LOQ fortification is 50 ng (calculated previously), the method dilution factor of 40, and the final extract volume of 5 mL, the in-solution concentration of analyte in the final extract at 100% recovery was:

$$(50 \text{ ng}) (1/40) (1/5 \text{ mL}) = 0.25 \text{ ng/mL}$$

A 1X LOQ working standard instrument calibration solution was prepared by bringing 12.5 μL of the 0.020 ng/ μL bifenazate stock standard to 1.0 mL in dilution solution:

$$[(12.5 \mu\text{L}) (0.020 \text{ ng}/\mu\text{L})] / (1 \text{ mL}) = 0.25 \text{ ng/mL}$$

Example calculations for a 1X LOQ standard for a mint oil analysis:

Taking into account that the total amount of analyte in an extract from a 1X LOQ fortification is 0.50 ng (calculated previously) and the final extract volume of 5 mL, the in-solution concentration of analyte in the final extract at 100% recovery was:

$$(0.50 \text{ ng}) (1/5 \text{ mL}) = 0.10 \text{ ng/mL}$$

A 1X LOQ working standard instrument calibration solution was prepared by bringing 5.0 μL of the 0.020 ng/ μL bifenazate stock standard to 1.0 mL in dilution solution:

$$[(5.0 \mu\text{L}) (0.020 \text{ ng}/\mu\text{L})] / (1 \text{ mL}) = 0.10 \text{ ng/mL}$$

The amounts of stock standards needed to prepare instrument calibration working standards at other concentrations were calculated in a similar manner.

Calculation of bifenazate concentrations

Bifenazate concentration values on the printouts were in $\mu\text{g/g}$ (ppm). When a sample required dilution because the peak area was not within the range of the standard curve, a sample dilution factor was used to correct the concentration of analyte in the sample. If dilution was not required, this factor was one. The quantitation values reported on the printouts included the method dilution factor (for mint tops analyses) and sample dilution factor, and the residues were calculated to four decimal places. Calculations of

the analyte concentrations were accomplished using the PE SCIEX Analyst Data System software by interpolation along the curve generated by the calibration standards analyzed with the sample set.

Bifenazate concentrations were calculated by the software using one of the following equations:

Linear regression curve:

$$Y = bX + a$$

where
Y = area counts for analyte
X = concentration of analyte
a = intercept constant from the linear regression
b = slope constant from the linear regression

Quadratic regression curve:

$$X = \frac{-b \pm \sqrt{b^2 - 4a(c - Y)}}{2a} * DF$$

where
Y = area counts for analyte
X = concentration of analyte
a, b, & c = coefficients from the quadratic regression
DF = sample dilution factor

Bifenazate residue results for experimental samples were reported to two significant figures. The analytical residue result from an experimental sample represented the combined total of bifenazate and UCC-D3598 residues in the sample.

Example linear regression calculation:

Bifenazate in mint tops sample M-01-02 (0109722-02A), extracted 10/22/01 and analyzed 10/23/01 (see Figure AA17 in Appendix A):

where
Y = 405421
a = -2.15E+003
b = 3.92E+06
DF = 200

$$X = \frac{Y - a}{b} * DF$$

$$\begin{aligned}
 X &= \frac{405421 - (-2.15E+003)}{3.92E+06} * 200 \\
 &= 20.7944 \mu\text{g/g (ppm)}^* \\
 &= 21 \mu\text{g/g (ppm)}
 \end{aligned}$$

Example quadratic regression calculation:

Bifenazate in mint oil sample M-01-02L (0109722-06A), extracted 10/23/01 and analyzed 10/23/01 (see Figure AA37 in Appendix A):

where

$$\begin{aligned}
 Y &= 195509 \\
 a &= -4.97E+05 \\
 b &= 1.3E+006 \\
 c &= 990 \\
 DF &= 10
 \end{aligned}$$

$$\begin{aligned}
 X &= \frac{-1.3E+006 \pm \sqrt{(1.3E+006)^2 - 4(-4.97E+05)(990 - 195509)}}{2(-4.97E+05)} * 10 \\
 &= 1.5934 \mu\text{g/g (ppm)}^* \\
 &= 1.6 \mu\text{g/g (ppm)}
 \end{aligned}$$

*NOTE: The coefficients and area counts used by the PE SCIEX Analyst Data System software to calculate residues included more places to the right of the decimal point than the numbers printed out in the data packages. Therefore, the final calculation cannot always be reproduced by hand beyond the second/third place to the right of the decimal. In the above examples, the software calculation gave residue concentration results of 20.8066 $\mu\text{g/g}$ and 1.5931 $\mu\text{g/g}$, while the hand calculations gave concentration results of 20.7944 $\mu\text{g/g}$ and 1.5934 $\mu\text{g/g}$, respectively.

Calculations for method fortification recoveries

Method fortification recovery (%) =

$$\frac{\text{residue } (\mu\text{g/g}) \text{ method fortification}}{\text{fortification concentration } (\mu\text{g/g})} \times 100\%$$

Example calculation for bifenazate on mint tops sample M-01-01 (0109722-01A), fortified at 1.0 $\mu\text{g/g}$, extracted 10/22/01 and analyzed 10/22/01 (see Figure AA11 in Appendix A):

$$\begin{aligned}
 \text{Method fortification recovery} &= \frac{0.9749 \mu\text{g/g}}{1.0 \mu\text{g/g}} \times 100\% \\
 &= 97.5\%
 \end{aligned}$$

STATISTICS STATEMENT

The mean percent recoveries were arithmetic means, and standard deviations for the mean percent recoveries were calculated and designated as σ_{n-1} , and expressed as an absolute percent value. The PE SCIEX Analyst Data System software calculated the linear or quadratic regression equation and the r-squared value of the standard calibration curve, and the analyte concentrations. The r-squared value for the standard calibration curve analyzed in each run was >0.995 . Sample analyte concentrations were calculated by interpolation along the standard curve, using the linear or quadratic regression equation generated by the calibration standards analyzed with the sample set.

Fortification percent recoveries were calculated using an Excel spreadsheet and then confirmed using a HP20S calculator. Mean percent recoveries were calculated and confirmed using a HP20S calculator. Analyte concentrations were verified using the coefficients and area counts printed in the data packages and a HP20S calculator (linear regression) or an Excel spreadsheet (quadratic regression). At minimum, two standards, one/two fortifications and two samples were verified for each sample set.

RESULTS

Method performance validation results for bifentazate and UCC-D3598 in/on mint tops are reported in Table 1. Over three fortification levels, bifentazate recoveries ranged from 75.8% to 107% and the mean percent recovery ($\pm \sigma_{n-1}$) was $94.3 \pm 8.6\%$; UCC-D3598 recoveries ranged from 92.1% to 114% and the mean percent recovery ($\pm \sigma_{n-1}$) was $100 \pm 9.0\%$.

Method performance validation results for bifentazate and UCC-D3598 in/on mint oil are reported in Tables 2A and 2B. Table 2A presents the results to the first mint oil validation set that was analyzed based on an LOQ of $0.050 \mu\text{g/g}$ (ppm) and Table 2B presents the results to the second mint oil validation set that was analyzed based on the lower LOQ of $0.010 \mu\text{g/g}$ (ppm). In the first validation set in/on mint oil (LOQ $0.050 \mu\text{g/g}$), and over three fortification levels, bifentazate recoveries ranged from 98.0% to 112% and the mean percent recovery ($\pm \sigma_{n-1}$) was $102 \pm 4.3\%$; UCC-D3598 in/on mint oil, and over three fortification levels, recoveries ranged from 80.2% to 97.0% and the mean percent recovery ($\pm \sigma_{n-1}$) was $91.1 \pm 6.5\%$. In the second validation in/on mint oil (LOQ $0.010 \mu\text{g/g}$), and over three fortification levels, bifentazate recoveries ranged from 92.0% to 107% and the mean percent recovery ($\pm \sigma_{n-1}$) was $100 \pm 4.7\%$; UCC-D3598 recoveries ranged from 92.0% to 106% and the mean percent recovery ($\pm \sigma_{n-1}$) was $101 \pm 5.4\%$. Over all six fortification levels, bifentazate recoveries ranged from 92.0% to 112% and the overall mean percent recovery ($\pm \sigma_{n-1}$) was $101 \pm 4.5\%$; UCC-D3598 recoveries ranged from 80.2% to 106% and the overall mean percent recovery ($\pm \sigma_{n-1}$) was $96.2 \pm 7.8\%$.

The analytical residue results for the mint tops and mint oil experimental samples are reported in Tables 3 and 4, respectively. The residue results are reported uncorrected for method recovery, or for any trace residues. The total bifentazate and UCC-D3598 residues in the three

treated mint tops samples were 21 $\mu\text{g/g}$, 21 $\mu\text{g/g}$, and 19 $\mu\text{g/g}$, and the total bifentazate and UCC-D3598 residues in the three treated mint oil samples were 1.6 $\mu\text{g/g}$, 0.90 $\mu\text{g/g}$, and 0.97 $\mu\text{g/g}$. No quantifiable residues were detected in either the untreated control mint tops sample or the untreated control mint oil sample.

Method fortification data for the one mint tops sample analysis are reported in Table 5. Recoveries from bifentazate fortifications in/on mint tops were 87.6% and 97.5% and recoveries from UCC-D3598 fortifications in/on mint tops were 89.8% and 95.7%.

Method fortification data for the three mint oil analyses are reported in Table 6. Recoveries from bifentazate fortifications in/on mint oil ranged from 98.2% to 121%, with a mean recovery ($\pm \sigma_{n-1}$) of $111 \pm 9.2\%$, and recoveries from UCC-D3598 fortifications in/on mint oil ranged from 90.0% to 120%, with a mean recovery ($\pm \sigma_{n-1}$) of $109 \pm 11\%$.

Chain of custody information is presented in Table 7. In all cases, analysis was conducted within 30 days of sampling.