## PURPOSE

This study was conducted to fulfill EPA requirements set forth in guideline OCSPP 850.6100 and PR Notice 96-1. This study provides validation data demonstrating that an independent researcher could reproduce the results of the analytical methods with minimal contact with the method developers.

## EXPERIMENTAL DESIGN

Soil and sediment were fortified with halosulfuron-methyl and its degradates, at two concentrations and analyzed according to three separate methods supplied by the Sponsor for each of three groups (HSM/RRE/CPSA/AP - Method 1, CSA/HS - Method 2, and CSAG/CSEG - Method 3). The limit of quantitation (LOQ) for HSM/RRE analytes only was set at 0.500 ppb. The LOQ for CPSA/AP/CSA/HS/CSAG/CSEG analytes was set at 1.00 ppb. The higher concentration was ten-fold the LOQ, i.e., 5.00 and 10.0 ppb, respectively. Reagent and matrix blanks (controls) were analyzed concurrently to evaluate potential analytical interferences.

## MATERIALS AND METHODS

### Untreated Control Soil and Sediment - Origin

Soil control matrix used for this study was provided by PTRL West in Hercules, CA (Sample I.D. – 2439W-074 – Original method validation matrix) and was assigned a USDA Textural Class description of sandy clay loam. Sediment control matrix used for this study was also provided by PTRL West in Hercules, CA (Sample I.D. – 2706W-018 – Original method validation matrix) and was assigned a USDA Textural Class description of sand. Both the soil and sediment control matrices were characterized by Agvise Laboratories, Inc. and summary characterization reports are presented in Appendix III. Upon receipt at EAG Laboratories testing facility, the soil and sediment samples were stored under refrigerated conditions.

### Analytical Reference Substances

A reference substance of halosulfuron-methyl was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification number 12764. The material was a solid and was identified on the label as halosulfuron-methyl (HSM); Lot# 110706; Purity 99.0%; CAS Number 100784-20-1; Expiration Date 07/06/2020. This reference substance was stored under ambient conditions. A certificate of analysis is presented in Appendix IV.

A reference substance of halosulfuron-methyl rearrangement ester was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification

number 12766. The material was a solid and was identified on the label as halosulfuron-methyl rearrangement ester (RRE); Lot# 035-030618-1; Purity 100%; Expiration Date 04/10/2019. This reference substance was stored under freezer conditions. A certificate of analysis is presented in Appendix IV.

A reference substance of 3-chlorosulfonamide acid methyl ester was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification number 12771. The material was a solid and was identified on the label as 3-chlorosulfonamide acid methyl ester (CPSA); Lot# CPSA-S931205; Purity 99.8%; CAS Number 100784-27-8; Expiration Date 08/25/2019. This reference substance was stored under refrigerated conditions. A certificate of analysis is presented in Appendix IV.

A reference substance of 2-amino-4,6-dimethoxypyrimidine was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification number 12770. The material was a solid and was identified on the label as amino-4,6-dimethoxypyrimidine (AP); Lot# SSDA309; Purity 100%; CAS Number 36315-01-2; Expiration Date 02/26/2019. This reference substance was stored under refrigerated conditions. A certificate of analysis is presented in Appendix IV.

A reference substance of halosulfuron acid was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification number 12769. The material was a solid and was identified on the label as halosulfuron acid (HS); Lot# 319ACID-S050331; Purity 99.9%; CAS Number 135397-30-7; Expiration Date 06/01/2020. This reference substance was stored under refrigerated conditions. A certificate of analysis is presented in Appendix IV.

A reference substance of 3-chlorosulfonamide acid was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification number 12765. The material was a solid and was identified on the label as 3-chlorosufonamide acid (CSA); Lot# CPSA-ACID-S9101; Purity 99.9%; Expiration Date 06/21/2016. This reference substance was stored under refrigerated conditions. A certificate of analysis is presented in Appendix IV.

A reference substance of halosulfuron guanidine was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification number 12767. The material was a solid and was identified on the label as halosulfuron acid guanidine (CSE-g); Lot# CSEG-S091224; Purity 92.9%; Expiration Date 05/07/2019. This reference substance was stored under refrigerated conditions. A certificate of analysis is presented in Appendix IV.

A reference substance of halosulfuron acid guanidine was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories Identification number 12768. The material was a solid and was identified on the label as halosulfuron guanidine (CSA-g); Lot# CSAG-S091224; Purity 97.5%; Expiration Date 05/07/2019. This reference substance was stored under refrigerated conditions. A certificate of analysis is presented in Appendix IV.

All eight reference substances above were used to prepare separate primary analytical stocks and subsequently various combined secondary fortification/calibration stocks and standards.

**Preparation of Primary Analytical Stocks and Secondary Combined Fortification Stocks and Calibration Standards**

**HSM/RRE/CPSA/AP (Method 1)**:

A primary stock solution of HSM reference standard was prepared by weighing a 25.25 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 0.500 mg/mL (corrected for purity).

A primary stock solution of RRE reference standard was prepared by weighing a 25.00 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 0.500 mg/mL.

A primary stock solution of CPSA reference standard was prepared by weighing a 25.05 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 0.500 mg/mL (corrected for purity).

A primary stock solution of AP reference standard was prepared by weighing a 25.00 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 0.500 mg/mL.

Separate combined secondary fortification/calibration stocks of HSM/RRE and of CPSA/AP analytes were prepared at 250 ng/mL and 500 ng/mL, respectively in acetonitrile as shown below:

| Primary Stock Conc. (mg/mL) | Aliquot (mL) | Final Volume (mL) | Combined Standard Conc. (ng/mL) |
|---|---|---|---|
| 500 (HSM) | 0.0500 | 100 | 250 |
| 500 (RRE) | 0.0500 | | |
| 500 (CPSA) | 0.100 | 100 | 500 |
| 500 (AP) | 0.100 | | |

All solutions were prepared using volumetric flasks and gas-tight syringes and were stored under freezer conditions when not in use.

Combined working calibration standards (HSM/RRE/CPSA/AP) ranging in concentration from 0.0500 to 25.0 ng/mL for HSM/RRE and from 0.100 to 50.0 ng/mL for CPSA/AP, were prepared in acetonitrile: HPLC grade water (1:1, v/v) from the combined secondary fortification stocks above as shown below:

| Combined Secondary Calibration Stock Concentration (HSM, RRE/CPSA, AP) (ng/mL) | Aliquot (mL) | Final Volume (mL) | Combined Calibration STD Conc. (HSM, RRE/CPSA, AP) (ng/mL) |
|---|---|---|---|
| 250/500 | 1.00/1.00 | 10.0 | 25.0/50.0 |
| 250/500 | 0.400/0.400 | 10.0 | 10.0/20.0* |
| 250/500 | 0.200/0.200 | 10.0 | 5.00/10.0 |
| 250/500 | 0.100/0.100 | 10.0 | 2.50/5.00 |
| 250/500 | 0.0400/0.0400 | 10.0 | 1.00/2.00 |
| 250/500 | 0.0200/0.0200 | 10.0 | 0.500/1.00 |
| 10.0/20.0 * | 0.250 | 10.0 | 0.250/0.500 |
| 10.0/20.0 * | 0.100 | 10.0 | 0.100/0.200 |
| 10.0/20.0 * | 0.0500 | 10.0 | 0.0500/0.100 |

*Note: 10.0/20.0 ng/mL combined calibration standard level was used to prepare three low-level calibration standards as shown.

Combined calibration standard solutions were transferred to amber bottles and stored under refrigerated conditions when not in use.

**CSA/HS (Method 2):**

A primary stock solution of CSA reference standard was prepared by weighing a 25.02 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 0.500 mg/mL (corrected for purity).

A primary stock solution of HS reference standard was prepared by weighing a 5.01 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 0.100 mg/mL (corrected for purity).

Combined secondary fortification/calibration stocks of CSA/HS analytes were prepared at 500 ng/mL in acetonitrile, and at 50.0 ng/mL in acetonitrile: HPLC grade water, (1:1, v/v) as shown below:

| Primary Stock Conc. (µg/mL) | Aliquot (mL) | Final Volume (mL) | Combined Standard Conc. (ng/mL) |
|---|---|---|---|
| 500 (CSA) | 0.100 | 100 | 500 |
| 100 (HS) | 0.500 | | |

All solutions were prepared using volumetric flasks and gas-tight syringes. The acetonitrile standards were stored under freezer conditions and the acetonitrile: HPLC grade water (1:1, v/v) were stored under refrigerated conditions when not in use.

Combined working calibration standards of CSA/HS ranging in concentration from 0.0400 to 50.0 ng/mL were prepared in acetonitrile: HPLC grade water (1:1, v/v) from the combined secondary fortification stock above as shown below:

| Combined Secondary Calibration Stock Concentration (CSA/HS) (ng/mL) | Aliquot (mL) | Final Volume (mL) | Combined Calibration STD Conc. (CSA/HS) (ng/mL) |
|---|---|---|---|
| 500 | 1.00 | 10.0 | 50.0 |
| 500 | 0.400 | 10.0 | 20.0 |
| 500 | 0.200 | 10.0 | 10.0* |
| 500 | 0.100 | 10.0 | 5.00 |
| 500 | 0.0400 | 10.0 | 2.00 |
| 500 | 0.0200 | 10.0 | 1.00 |
| 10.0* | 0.500 | 10.0 | 0.500 |
| 10.0* | 0.200 | 10.0 | 0.200 |
| 10.0* | 0.100 | 10.0 | 0.100 |
| 10.0* | 0.0400 | 10.0 | 0.0400 |

*Note: 10.0 ng/mL combined calibration standard level was used to prepare four low-level calibration standards as shown.

Combined calibration standard solutions were transferred to amber vials and stored under refrigerated conditions when not in use.

**CSAG/CSEG (Method 3):**

A primary stock solution of CSAG reference standard was prepared by weighing a 25.64 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile: HPLC grade water (1:1, v/v) dilution solvent to yield a final nominal stock concentration of 0.500 mg/mL (corrected for purity).

A primary stock solution of CSEG reference standard was prepared by weighing a 26.91 mg aliquot into a vial. The reference material was dissolved, transferred to a 50-mL volumetric flask, and adjusted to final volume using acetonitrile: HPLC grade water (1:1, v/v) dilution solvent to yield a final nominal stock concentration of 0.500 mg/mL (corrected for purity).

Separate secondary stocks of CSAG and CSEG analytes were prepared using appropriate dilution of the primary stocks at 1000 ng/mL in acetonitrile: HPLC grade water (1:1, v/v) dilution solvent as shown below:

| Primary Stock Conc. (µg/mL) | Aliquot (mL) | Final Volume (mL) | Secondary Stock Conc. (ng/mL) |
|---|---|---|---|
| 500 (CSAG) | 0.100 | 50.0 | 1000 |
| 500 (CSEG) | 0.100 | 50.0 | 1000 |

The 1000 ng/mL secondary stocks were used in the preparation of working calibration standards and in the fortification of the method validation recovery samples.

All stock solutions were prepared using volumetric flasks and gas-tight syringes and were stored under refrigerated conditions when not in use.

Combined working calibration standards of CSAG/CSEG ranging in concentration from 0.0400 to 10.0 ng/mL were prepared in acetonitrile: HPLC grade water (1:1, v/v) dilution solvent from the separate 1000 secondary stocks of each analyte prepared above as shown below:

| Separate Secondary Calibration Stock Concentration (CSAG/CSEG (ng/mL) | Aliquot (mL) | Final Volume (mL) | Combined Calibration Conc. (CSAG/CSEG) (ng/mL) |
|---|---|---|---|
| 1000/1000 | 0.100/0.100 | 10.0 | 10.0* |
| 1000/1000 | 0.0500/0.0500 | 10.0 | 5.00 |
| 1000/1000 | 0.0200/0.0200 | 10.0 | 2.00 |
| 10.0/10.0 | 1.00 | 10.0 | 1.00 |
| 10.0/10.0* | 0.500 | 10.0 | 0.500 |
| 10.0/10.0* | 0.200 | 10.0 | 0.200 |
| 10.0/10.0* | 0.100 | 10.0 | 0.100 |
| 10.0/10.0* | 0.0400 | 10.0 | 0.0400 |

*Note: 10.0 ng/mL combined calibration standard level was used to prepare five low-level calibration standards as shown.

Combined calibration standard solutions were transferred to amber vials and stored under refrigerated conditions when not in use.

**Analytical Methods – Soil/Sediment**

Three separate residue analytical methods were developed for soil/sediment matrices and provided for validation for this Independent Laboratory Validation (ILV) study. Method 1 was used for HSM/RRE/CPSA/AP analytes, Method-2 was used for CSA/HS analytes, and Method-3 was used for CSAG/CSEG analytes. Method 1 employed a shaker table extraction, followed by a liquid-liquid partitioning procedure, while Method-2 and Method-3 were based upon the principles of the QuEChERS approach. Final quantitation of samples was performed utilizing High Performance Liquid Chromatography with tandem mass spectrometric detection (HPLC/MS/MS).

**Fortification of Recovery Samples**

For each of the three methods validated, one reagent blank, two unfortified matrix blanks, five fortified control matrix samples at the LOQ, and five fortified control matrix samples at 10X the LOQ were prepared in soil and sediment as shown below for each of the three analytes groups:

HSM/RRE/CPSA/AP (Method-1)

| Analyte(s) | Nominal Concentration. (ppb) | Fortification Volume (mL) | Sample Weight (g) | Combined Stock Conc. (ng/mL) |
|---|---|---|---|---|
| HSM/RRE | 0. 500(LOQ) | 0.0200 | 10.0 | 250 |
| | 5.00(10x LOQ) | 0.200 | 10.0 | 250 |
| CPSA/AP | 1.00(LOQ) | 0.0200 | 10.0 | 500 |
| | 10.0(10x LOQ) | 0.200 | 10.0 | 500 |

CSA/HS (Method-2)

| Analyte (s) | Nominal Concentration. (ppb) | Fortification Volume (mL) | Sample Weight (g) | Combined Stock Conc. (ng/mL) |
|---|---|---|---|---|
| CSA/HS | 1.00(LOQ) | 0.0200 | 10.0 | 500 |
| | 10.0(10x LOQ) | 0.200 | 10.0 | 500 |

CSAG/CSEG (Method-3)

| Analyte (s) | Nominal Concentration. (ppb) | Fortification Volume (mL) | Sample Weight (g) | Separate Stock Conc. (ng/mL) |
|---|---|---|---|---|
| CSAG | 1.00(LOQ) | 0.0100 | 10.0 | 1000 |
| | 10.0(10x LOQ) | 0.100 | 10.0 | 1000 |
| CSEG | 1.00(LOQ) | 0.0100 | 10.0 | 1000 |
| | 10.0(10x LOQ) | 0.100 | 10.0 | 1000 |

All fortified samples were prepared with fortification solutions that were prepared compensating for the purity of the reference materials. Therefore, residue fortification and recovery levels, expressed in ppb, are equivalent to the expression as ppb active ingredient (ppb a.i.).

**Method 1 - Extraction and Analysis of HSM/RRE/CPSA/AP from Soil/Sediment**

For analysis, 10.0-gram aliquots of control soil/sediment were weighed into twelve individually labeled 50-mL graduated plastic disposable centrifuge tubes. Two grams of celite and 3.3 grams of sand were also added to each tube and mixed. Following, five of the samples were fortified at the LOQ (0.500 ppb-HSM/RRE; 1.00 ppb-CPSA/AP) and five at 10X the LOQ (5.00 ppb-HSM/RRE; 10.0 ppb-CPSA/AP) with combined secondary fortification stocks of the reference substances prepared as described above. A single reagent blank consisting of all reagents except matrix, and the two matrix blanks of unfortified control matrix and any reagents were also prepared and carried through the methodology for each matrix. All samples were subsequently analyzed by methodology in Appendix II. Slight deviations in the LC/MS/MS

source optimization parameters were utilized and were considered to be equivalent values related to inherent differences in instrumental performance and not a limitation of the methodology. Since specific details of the method are presented in Appendix II, a more general description is provided here.

Twenty milliliters (20 mL) of acetonitrile (ACN) and 5.0 mL of deionized water were added to each validation sample and the set was placed on a shaker where the tubes were shaken for approximately 10 minutes at a setting of 300 excursions/minute. The extracted samples were centrifuged at approximately 3000 RPM for approximately 5 minutes and the supernatants were filtered through a Whatman No. 4 filter paper contained in a fritted funnel apparatus into an appropriate sized flask with the aid of slight vacuum. The soil plugs for each sample were broken up, and the ACN/water extraction, centrifugation, and filtration procedure above was repeated, combining the extracts in the same flasks. The soil plugs were broken up again and the extraction shake, centrifugation, and filtration procedure was repeated two additional times using 20 mL of dichloromethane (DCM) solvent with each extraction. The extracts were combined each time in the same flasks from above. The sample tubes and filter cake were rinsed with approximately 10 mL of DCM following the last processing of the samples. The combined final filtrates were transferred to 250-mL separatory funnels. Each of the collection flasks was subsequently rinsed and sonicated with approximately 30mL of DCM and combined in the separatory funnels with the filtrates. The separatory funnels were shaken vigorously by hand for approximately 2 minutes and the phases allowed to separate (~ 5 minutes). The lower DCM layers were passed through filter funnels containing approximately 5.5 g of sodium sulfate into 250-mL round-bottom flasks and the filter funnels were subsequently rinsed with 10 mL of DCM. Thirty (30 mL) volumes of ethyl acetate (EtoAc) extraction solvent were next added to the aqueous layer in each of the funnels and the mixtures were shaken as above. After phase separation (~ 5 minutes) the lower aqueous layer was drained into a suitable waste container and the upper organic layer (EtoAc) was passed through the sodium sulfate filter funnels and combined into the same 250-mL concentration flasks. The separatory and filter funnels were rinsed with 5 and 10 mL volumes of ethyl acetate, respectively. Each of the combined extracts was then evaporated by rotary-evaporation at ~30°C to a volume of approximately 5 mL. The reduced extracts were filtered through Whatman 0.2 µm syringeless filter assemblies into a disposable 15-mL glass tubes. The round-bottom flasks and syringeless filters were rinsed with approximately 5 mL of EtoAc and into their corresponding tubes. These extracts were reduced to near dryness (0.200 to 0.400 mL) using a nitrogen evaporator at approximately 30 °C and then to complete dryness using a gentle stream of nitrogen and a disposable pasteur pipet. (Note: care was taken to minimize the amount of time sample residues were dry in tube). The final residues were reconstituted in 2.00 mL volume of acetonitrile: HPLC grade water (1:1, v/v) and vortexed well. Additionally, the final extracts were placed in a sonication water bath for approximately 5 minutes as an aid to further ensure adequate dissolving of residues. The final extracts were then diluted, as necessary, using acetonitrile: HPLC grade water (1:1, v/v), followed by transfer to auto-sampler vials and submission for LC/MS/MS analysis.

**Method 2 - Extraction and Analysis of CSA/HS from Soil/Sediment**

For analysis, 10.0 gram aliquots of control soil/sediment were measured into twelve individually labeled 50-mL plastic centrifuge tubes, five of which were fortified at the LOQ (1.00 ppb) and five at 10x the LOQ (10.0 ppb) with combined secondary fortification stocks of the reference substances prepared as described above. A single reagent blank consisting of all reagents except matrix, and the two matrix blanks of unfortified control matrix and any reagents were also prepared and carried through the methodology for each matrix. All samples were subsequently analyzed by methodology presented in Appendix II. Slight deviations in the LC/MS/MS source optimization parameters were utilized and were considered to be equivalent values related to inherent differences in instrumental performance and not a limitation of the methodology. Since specific details of the method are presented in Appendix II, a more general description is provided here.

Ten milliliters (10.0 mL) of acetonitrile: 1% acetic acid, 5.0 mL of HPLC grade water, and two 8mm stainless steel (SS) grinding balls were added to each of the sample tubes. The samples were placed on a SPEX GenoGrinder sample processor at a setting of 1500 RPM for approximately two minutes. Following, a single package of Restek Q100 un-buffered extraction salts (1 g NaCl, 4 g $MgSO_4$) was added to each tube. The sample tubes were shaken on a shaker table at setting of approximately 300 excursions/ minute for five minutes and then centrifuged at approximately 3000 rpm for approximately five minutes. An aliquot of the final acetonitrile solvent layers were filtered using 0.45 µm nylon micro-centrifuge filters into amber storage vials and to auto-sampler vials and submitted for LC/MS/MS analysis. The remainders of each sample acetonitrile solvent layer were transferred to amber vials for storage.

**Method 3 - Extraction and Analysis of CSAG/CSEG from Soil/Sediment**

For analysis, 10.0 gram aliquots of control soil/sediment were measured into twelve individually labeled 50-mL plastic centrifuge tubes, five of which were fortified at the LOQ (1.00 ppb) and five at 10x the LOQ (10.0 ppb) with separate secondary fortification stocks of the reference substances prepared as described above. A single reagent blank consisting of all reagents except matrix, and the two matrix blanks of unfortified control matrix and any reagents were also prepared and carried through the methodology for each matrix. All samples were subsequently analyzed by methodology presented in Appendix II. Slight deviations in the LC/MS/MS source optimization parameters were utilized and were considered to be equivalent values related to inherent differences in instrumental performance and not a limitation of the methodology. Since specific details of the method are presented in Appendix II, a more general description is provided here.

Five milliliters (5.00 mL) of acetonitrile:1% acetic acid, 5.00 mL of HPLC grade water, and two 8 mm SS grinding balls were added to each of the sample tubes. The samples were shaken on a SPEX GenoGrinder sample processor at a setting of 1500 RPM for approximately two minutes and then centrifuged at approximately 4000 RPM for approximately ten minutes. The soil extracts were decanted into individual 50-mL graduated cylinders. After breaking up the soil plug in the extraction tubes, the above extraction procedure was repeated. The samples tubes were centrifuged at approximately 4000 RPM for approximately five minutes and the soil extracts were combined with the first extracts in the 50-mL graduated cylinders. The centrifuge

tubes were rinsed and the graduated cylinders were brought to 30mL final volume using a solution of acetonitrile HPLC grade water (1:1, v/v). The final (30 mL) extracts were transferred to new 50-mL disposable plastic centrifuge tubes. One milliliter (1.0 mL) of concentrated hydrochloric acid, a single package of Restek Q100 un-buffered extraction salts (1 g NaCl, 4 g $MgSO_4$) and two 8mm stainless steel (SS) grinding balls were added to each tube. The sample tubes were shaken on a SPEX GenoGrinder sample processor for 2 minutes at a setting of 1500 rpm and then centrifuged at 3000 rpm for approximately five minutes. The solvent layers were transferred to amber vials for storage. An aliquot of the final acetonitrile solvent layers were filtered using a 0.22 µm micro-centrifuge filters and transferred to auto-sampler vials and submitted for LC/MS/MS analysis. The remainders of each sample acetonitrile solvent layer were transferred to amber vials for storage.

**Quantitation of HSM/RRE/CPSA/AP, CSA/HS, and CSAG/CSEG by LC/MS/MS**

An Agilent Technologies Model 1260 High Performance Liquid Chromatograph connected to an AB Sciex Triple Quad 5500 Mass Spectrometric Detector (LC/MS/MS) was used to analyze samples. An acidified (0.1% formic acid) acetonitrile: water gradient was used.

Quantitation was performed using the responses of the primary ion transitions for each analyte. Confirmation analysis was performed using the responses of the secondary confirmation ion transitions for each analyte as well. The ion transitions monitored are summarized below:

| Analyte | Primary (Quantitation) | Secondary (Confirmation) |
|---|---|---|
| HSM | 435→182 amu | 435→139 amu |
| RRE | 328→296 amu | 328→197 amu |
| CPSA | 252→188 amu | 252→220 amu |
| AP | 156→100 amu | 156→57.0 amu |
| CSA | 238→78.0 amu | 238→194 amu |
| HS | 419→194 amu | 419→238 amu |
| CSAG | 323→194 amu | 323→238 amu |
| CSEG | 337→252 amu | 337→77.9 amu |

Specific details of the LC/MS/MS instrumentation and operational parameters are presented in Tables 1-3.

**Example Calculations**

For each analyte, a regression equation was derived from the chromatographic peak area responses of the analytes determined in calibration standard solutions versus the respective nominal concentrations of the standards. Standard curves were generated by plotting this function with analyte concentration (ng/mL) on the abscissa and the respective analyte peak area response on the ordinate. The applied regression was weighted 1/x with respect to concentration and expressed as a linear regression as follows:

$$y = mx + b$$

Where:
Y = peak area
m = slope
b = Y-intercept
x = analyte concentration

Concentrations of analytes in the samples (quantitation and confirmation analyses) were determined by substituting peak area responses of the samples into the re-arranged weighted (1/x) regression equation as follows:

$$\text{Analyte Concentration} = \frac{\text{Peak area - (Y-intercept)}}{\text{Slope}}$$

Using the data from the soil method validation sample 334C-132-SVMAS-1, 0.500 ppb shown below, the analytical result and percent recovery was calculated as follows using the software algorithms of Analyst Version 1.6.2 of the AB Sciex Triple Quad 5500 mass spectrometer system in full precision mode. Note: manual calculations shown here may differ slightly than reported.

Where:

Peak area = 13537.5
Y-intercept = 633.196
Slope = 50313.2

The concentration of HSM at instrument was determined by substituting the resulting analyte peak area response into the above equation. Using the values above, the concentration in the final sample solution was calculated as:

$$\text{Concentration at instrument (ng/mL): } = \frac{13537.5 - (633.196)}{50313.2}$$

Concentration at instrument (ng/mL): = 0.25648

The residue concentration (ppb) for HSM in the fortified soil recovery sample was determined as the product of the at instrument solution concentration determined above and the overall dilution factor as follows:

$$\text{Concentration in ppb} = \text{HSM Concentration at Instrument} \times \frac{\text{(Final Volume)}}{\text{(Initial Volume)}} \times \text{DF}$$

Where: Initial Weight= 10.0 g
Final Volume = 2.00 mL
Secondary Dilution (DF) = 10.0
ng/g = ppb

Using the nominal concentration (ng/mL) from above, the concentration of HSM in soil sample was calculated as follows:

Concentration in sample (ppb) = 0.25648 x 0.200 x 10.0

Concentration in sample (ppb) = 0.51296

The percent recovery was determined by dividing the concentration of the analyte recovered in the fortified sample by the nominal concentration added as shown below:

$$\text{Recovery (\%)} = \frac{\text{ppb Found}}{\text{ppb Added}} \times 100$$

For the above 0.500- ppb fortified sample, the percent recovery of HSM was calculated as:

$$\text{Recovery (\%)} = \frac{0.51296 \text{ ppb Found}}{0.500 \text{ ppb Added}} \times 100$$

Recovery (%) = 103%

The same calculation procedure was applied for the quantitation and confirmation analyses of HSM/RRE/CPSA/AP, CSA/HS, and CSAG/CSEG analytes for this study as well.

**Statistical Treatment of Data**

Mean recoveries for each analyte for each fortification level were calculated by dividing the sum of the percent recoveries by the total number of fortified samples. The standard deviation and relative standard deviation (coefficient of variation) for the recoveries for each analyte were also determined and reported for both quantitation and confirmation analyses.

Table 1. LC/MS/MS Instrumentation and Operational Parameters (HSM/RRE/CPSA/AP)

| | |
|---|---|
| Instrumentation | Agilent Technologies Model 1200 Series High Performance Liquid Chromatograph with a AB Sciex Triple QUAD 5500 Mass Spectrometric Detector (LC/MS/MS) and Turbo-V Ion Spray Source |
| Analytical Column | PHENOMENEX Synergi™ 4 µm Hydro-RP 80A (75 mm x 2.0 mm) |
| Guard Column | PHENOMENEX Security Guard Cartridge System-Aqueous C18 (4 x 2 mm) |
| Mobile Phases | A1: 0.1% Formic Acid in HPLC-grade water<br>B1: 0.1% Formic Acid in Acetonitrile<br>Gradient Elution Program:<br>Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C)<br>0.00 / 100 / 0.00 / 500 / 30.0<br>1.00 / 100 / 0.00 / 500 / 30.0<br>5.00 / 0.00 / 100 / 500 / 30.0<br>9.00 / 0.00 / 100 / 500 / 30.0<br>9.50 / 100 / 0.00 / 500 / 30.0<br>20.0 / 100 / 0.00 / 500 / 30.0 |
| Diverter Valve (Valco) | Time (min) / Position<br>0.00 / B |
| Injection Volume | 5.00 µL |
| Total Run Time | 20.0 minutes |
| Period 1-Experiment 1<br>**AP** | Scan Type/Polarity: MRM/Positive: GS1 = 50, GS2 = 70, CUR = 40.0, CAD = 9, IS = 5500, TEM = 500, DP = 115, EP = 10<br>Quantitation: (252/188 amu), CE = -18, CXP = -15<br>Confirmation: (252/220 amu), CE = -20, CXP = -19<br>Retention Time: Approximately 5.0 minutes |
| Period 1-Experiment 2<br>**CPSA** | Scan Type/Polarity: MRM/Negative: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = -4500, TEM = 500, DP = -95, EP = -10<br>Quantitation: (156/100 amu), CE = 29, CXP = 12<br>Confirmation: (156/57.0 amu), CE = 29, CXP = 8.0<br>Retention Time: Approximately 5.8 minutes |
| Period 1-Experiment 3<br>**RRE**<br><br>**HSM** | Scan Type/Polarity: MRM/Positive: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = 5500, TEM = 500, EP = -10<br>Quantitation: (328/296 amu), DP = 110, CE = 27, CXP = 22<br>Confirmation: (328/197 amu), DP = 110, CE = 43, CXP = 22<br>Retention Time: Approximately 6.3 minutes<br>Quantitation: (156/100 amu), CE = 29, CXP = 12<br>Confirmation: (156/57.0 amu), CE = 29, CXP = 8.0<br>Retention Time: Approximately 6.4 minutes |

Table 2. LC/MS/MS Instrumentation and Operational Parameters (CSA/HS)

| | |
|---|---|
| Instrumentation | Agilent Technologies Model 1200 Series High Performance Liquid Chromatograph with a AB Sciex Triple QUAD 5500 Mass Spectrometric Detector (LC/MS/MS) and Turbo-V Ion Spray Source |
| Analytical Column | PHENOMENEX Synergi™ 4 µm Hydro-RP 80A (75 mm x 2.0 mm) |
| Guard Column | PHENOMENEX Security Guard Cartridge System-Aqueous C18 (4 x 2 mm) |
| Mobile Phases | A1: 0.1% Formic Acid in HPLC-grade water<br>B1: 0.1% Formic Acid in Acetonitrile<br>Gradient Elution Program:<br>Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C)<br>0.00 / 100 / 0.00 / 500 / 30.0<br>1.00 / 100 / 0.00 / 500 / 30.0<br>5.00 / 0.00 / 100 / 500 / 30.0<br>9.00 / 0.00 / 100 / 500 / 30.0<br>9.50 / 100 / 0.00 / 500 / 30.0<br>20.0 / 100 / 0.00 / 500 / 30.0 |
| Diverter Valve (Valco) | Time (min) / Position<br>0.00 / B |
| Injection Volume | 5.00 µL |
| Total Run Time | 20.0 minutes |
| Period 1-Experiment 1<br>**CSA** | Scan Type/Polarity: MRM/Negative: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = -4500, TEM = 500, EP = -10<br>Quantitation: (238/78.0 amu), DP = -40, CE = -31, CXP = -7<br>Confirmation: (238/194 amu), DP = -40, CE = -16, CXP = -9<br>Retention Time: Approximately 5.0 minutes |
| **HS** | Quantitation: (419/194 amu), DP = -60, CE = -30, CXP = -15<br>Confirmation: (419/238 amu), DP = -60 CE = -14, CXP = -17<br>Retention Time: Approximately 5.8 minutes |

Table 3. LC/MS/MS Instrumentation and Operational Parameters (CSAG/CSEG)

| | |
|---|---|
| Instrumentation | Agilent Technologies Model 1200 Series High Performance Liquid Chromatograph with a AB Sciex Triple QUAD 5500 Mass Spectrometric Detector (LC/MS/MS) and Turbo-V Ion Spray Source |
| Analytical Column | PHENOMENEX Synergi™ 4 µm Hydro-RP 80A (75 mm x 2.0 mm) |
| Guard Column | PHENOMENEX Security Guard Cartridge System-Aqueous C18 (4 x 2 mm) |
| Mobile Phases | A1: 0.1% Formic Acid in HPLC-grade water<br>B1: 0.1% Formic Acid in Acetonitrile<br>Gradient Elution Program:<br>Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C)<br>0.00 / 100 / 0.00 / 500 / 30.0<br>1.00 / 100 / 0.00 / 500 / 30.0<br>5.00 / 0.00 / 100 / 500 / 30.0<br>9.00 / 0.00 / 100 / 500 / 30.0<br>9.50 / 100 / 0.00 / 500 / 30.0<br>20.0 / 100 / 0.00 / 500 / 30.0 |
| Diverter Valve (Valco) | Time (min) / Position<br>0.00 / B |
| Injection Volume | 5.00 µL |
| Total Run Time | 20.0 minutes |
| Period 1-Experiment 1<br>**CSAG** | Scan Type/Polarity: MRM/Negative: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = -4500, TEM = 500, EP = -10<br>Quantitation: (323/194 amu), DP = -35, CE = -26, CXP = -19<br>Confirmation: (323/238 amu), DP = -35, CE = -14, CXP = -27<br>Retention Time: Approximately 5.0 minutes |
| **CSEG** | Quantitation: (337/252 amu), DP = -120, CE = -22, CXP = -23<br>Confirmation: (337/77.9 amu), DP = -120 CE = -38, CXP = -9<br>Retention Time: Approximately 5.2 minutes |