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

Ground and Surface water 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 0.0500 ppb. The LOQ for CPSA/AP/CSA/HS/CSAG/CSEG was set at 0.200 ppb. The higher concentration was ten-fold the LOQ, i.e., 0.500 and 2.00 ppb, respectively. Reagent and matrix blanks (controls) were analyzed concurrently to evaluate potential analytical interferences.

MATERIALS AND METHODS

Untreated Control Ground and Surface Water - Origin

Ground well water control matrix used for this study was collected by PTRL West in Hercules, CA (Sample I.D. - 2706-032 – Original method validation matrix) and also obtained locally from EAG Laboratories aquatic testing facility in Easton, MD. Both sources of water were stored under refrigerated conditions in the dark following collection and when not in use. The PTRL West ground water was characterized by Agvise Laboratories, Inc. and a summary report is presented in Appendix III. The EAG Laboratories ground well water was characterized internally and the mean results for the 4 week period immediately preceding its use is summarized to Appendix IV.

Surface water control matrix was obtained from a local source, Tuckahoe Lake located in Tuckahoe State Park, Ridgely, MD. The water was collected on March 29, 2016 and was logged in and stored under refrigerated conditions at the testing facility upon receipt. A summary of surface water characterization results is presented in Appendix V.

Analytical Reference Substances

A reference substance of halosulfuron-methyl was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories-Easton 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 07/06/2016. This reference substance was stored under ambient conditions. A certificate of analysis is presented in Appendix VI.

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

A reference substance of 3-chlorosulfonamide acid methyl ester was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories-Easton 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 VI.

A reference substance of 2-amino-4,6-dimethoxypyrimidine was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories-Easton 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 VI.

A reference substance of halosulfuron acid was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories-Easton 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 VI.

A reference substance of 3-chlorosulfonamide acid was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories-Easton 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 VI.

A reference substance of halosulfuron guanidine was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories-Easton 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 VI.

A reference substance of halosulfuron acid guanidine was received from Gowan/Nissan on December 15, 2015 and was assigned the EAG Laboratories-Easton 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 VI.

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 solvent 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		
0.500 (CSA/HS)	2.50	25.0	50.0

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 and 20.0 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
Secondary Stock Conc. (ng/mL)	**Aliquot (mL)**	**Final Volume (mL)**	**Secondary Stock Conc. (ng/mL)**
1000 (CSAG)	2.00	100	20.0
1000 (CSEG)	2.00	100	20.0

The 1000 and 20.0 ng/mL secondary stocks were used in the preparation of working calibration standards and the 20.0 ng/mL secondary stock was 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 and 20.0 ng/mL separate 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
20.0/20.0	0.500/0.500	10.0	1.00
20.0/20.0	0.250/0.250	10.0	0.500
20.0/20.0	0.100/0.100	10.0	0.200
20.0/20.0	0.0500/0.0500	10.0	0.100
20.0/20.0	0.0200/0.0200	10.0	0.0400

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

Analytical Methods – Ground/Surface Water

Three separate residue analytical methods were developed for ground/surface water 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 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 ground and surface water 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 Volume (mL)	Combined Stock Conc. (ng/mL)
HSM/RRE	0.0500(LOQ)	0.0200	100	250
	0.500 (10X LOQ)	0.200	100	250
CPSA/AP	0.200 (LOQ)	0.0400	100	500
	2.00(10X LOQ)	0.400	100	500

CSA/HS (Method-2)

Analyte(s)	Nominal Concentration (ppb)	Fortification Volume (mL)	Sample Volume (mL)	Combined Stock Conc. (ng/mL)
CSA/HS	0.200(LOQ)	0.0400	10.0	50.0
	2.00(10X LOQ)	0.400	10.0	50.0

CSAG/CSEG (Method-3)

Analyte(s)	Nominal Concentration (ppb)	Fortification Volume (mL)	Sample Volume (mL)	Separate Stock Conc. (ng/mL)
CSAG	0.200(LOQ)	0.100	10.0	20.0
	2.00(10X LOQ)	1.00	10.0	20.0
CSEG	0.200(LOQ)	0.100	10.0	20.0
	2.00(10X LOQ)	1.00	10.0	20.0

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 Ground/Surface Water

For analysis, 100-mL volumes of control ground/surface water were measured into twelve individually labeled 250-mL separatory funnels, five of which were fortified at the LOQ (0.0500 ppb-HSM/RRE; 0.200 ppb-CPSA/AP) and five at 10x the LOQ (0.500 ppb-HSM/RRE; 2.00 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.

Forty milliliters (40 mL) of dichloromethane (DCM) extraction solvent and approximately 1 gram of sodium chloride were added to each separatory funnel. The funnels were shaken vigorously for approximately 2 minutes and the phases allowed to separate for approximately 5 minutes. The lower organic layers (DCM) were drained through filter funnels containing approximately 5.5 g of sodium sulfate into 250-mL concentration flasks and the filter funnels were subsequently rinsed with approximately 10 mL of DCM. Forty (40 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 (~ 3 minutes) the lower aqueous layer was drained into a suitable waste container and the upper organic layer (EtoAc) was drained through the sodium sulfate filter funnels and combined into the same 250-mL concentration flasks. The separatory funnels and filter funnels were rinsed with approximately 5 mL of EtoAc into the concentration flasks. Each of the combined extracts were evaporated by rotary-evaporation at ~30°C to a volume of approximately 5 mL, and following quantitative transfer to 15-mL tubes using 5 mL volume of EtoAc as rinse of flasks, were reduced to near dryness (0.200 to 0.400 mL) using a nitrogen evaporator and then to complete dryness manually using a gentle stream of nitrogen. (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 Ground/Surface Water

For analysis, 10.0-mL volumes of control ground/surface water were measured into twelve individually labeled 50-mL plastic centrifuge tubes, five of which were fortified at the LOQ (0.200 ppb) and five at 10X the LOQ (2.00 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 extraction solvent were added to each of the sample tubes, followed by 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. 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 5 minutes. An aliquot of the final acetonitrile solvent layers were 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.

Method 3 - Extraction and Analysis of CSAG/CSEG from Ground/Surface Water

For analysis, 10.0-mL volumes of control ground/surface water were measured into twelve individually labeled 50-mL plastic centrifuge tubes, five of which were fortified at the LOQ (0.200 ppb) and five at 10X the LOQ (2.00 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.

Ten milliliters (10.0 mL) of acetonitrile extraction solvent and 1.0 mL of concentrated hydrochloric acid (HCl) were added to each of the sample tubes, followed by 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. 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 5 minutes. An aliquot of the final acetonitrile solvent layers were 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 ground water method validation sample 334C-131-GWVMAS-51, 0.0500 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 = 12216.5
Y-intercept = 425.391
Slope = 54555.6

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:

$$\text{Concentration at instrument (ng/mL): } = \frac{12216.5 - (425.391)}{54555.6}$$

Concentration at instrument (ng/mL): = 0.21613

The residue concentration (ppb) for HSM in the fortified water 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 Volume= 100 mL
Final Volume = 2.00 mL
Secondary Dilution (DF) = 10.0
ng/mL = ppb

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

Concentration in sample (ppb) = 0.21613 x 0.0200 x 10.0

Concentration in sample (ppb) = 0.04323

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 0.0500- ppb fortified sample, the percent recovery of HSM was calculated as:

$$\text{Recovery (\%)} = \frac{0.04323 \text{ ppb Found}}{0.0500 \text{ ppb Added}} \times 100$$

$$\text{Recovery (\%)} = 86.5\%$$

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 B1: 0.1% Formic Acid in Acetonitrile Gradient Elution Program: Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C) 0.00 / 100 / 0.00 / 500 / 30.0 1.00 / 100 / 0.00 / 500 / 30.0 5.00 / 0.00 / 100 / 500 / 30.0 9.00 / 0.00 / 100 / 500 / 30.0 9.50 / 100 / 0.00 / 500 / 30.0 20.0 / 100 / 0.00 / 500 / 30.0
Diverter Valve (Valco)	Time (min) / Position 0.00 / B
Injection Volume	5.00 µL
Total Run Time	20.0 minutes
Period 1-Experiment 1 **AP**	Scan Type/Polarity: MRM/Positive: GS1 = 50, GS2 = 70, CUR = 40.0, CAD = 9, IS = 5500, TEM = 500, DP = 115, EP = 10 Quantitation: (252/188 amu), CE = -18, CXP = -15 Confirmation: (252/220 amu), CE = -20, CXP = -19 Retention Time: Approximately 5.0 minutes
Period 1-Experiment 2 **CPSA**	Scan Type/Polarity: MRM/Negative: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = -4500, TEM = 500, DP = -95, EP = -10 Quantitation: (156/100 amu), CE = 29, CXP = 12 Confirmation: (156/57.0 amu), CE = 29, CXP = 8.0 Retention Time: Approximately 5.8 minutes
Period 1-Experiment 3 **RRE** **HSM**	Scan Type/Polarity: MRM/Positive: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = 5500, TEM = 500, EP = -10 Quantitation: (328/296 amu), DP = 110, CE = 27, CXP = 22 Confirmation: (328/197 amu), DP = 110, CE = 43, CXP = 22 Retention Time: Approximately 6.3 minutes Quantitation: (156/100 amu), CE = 29, CXP = 12 Confirmation: (156/57.0 amu), CE = 29, CXP = 8.0 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 B1: 0.1% Formic Acid in Acetonitrile Gradient Elution Program: Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C) 0.00 / 100 / 0.00 / 500 / 30.0 1.00 / 100 / 0.00 / 500 / 30.0 5.00 / 0.00 / 100 / 500 / 30.0 9.00 / 0.00 / 100 / 500 / 30.0 9.50 / 100 / 0.00 / 500 / 30.0 20.0 / 100 / 0.00 / 500 / 30.0
Diverter Valve (Valco)	Time (min) / Position 0.00 / B
Injection Volume	5.00 µL
Total Run Time	20.0 minutes
Period 1-Experiment 1 **CSA** **HS**	Scan Type/Polarity: MRM/Negative: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = -4500, TEM = 500, EP = -10 Quantitation: (238/78.0 amu), DP = -40, CE = -31, CXP = -7 Confirmation: (238/194 amu), DP = -40, CE = -16, CXP = -9 Retention Time: Approximately 5.0 minutes Quantitation: (419/194 amu), DP = -60, CE = -30, CXP = -15 Confirmation: (419/238 amu), DP = -60 CE = -14, CXP = -17 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 B1: 0.1% Formic Acid in Acetonitrile Gradient Elution Program: Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C) 0.00 / 100 / 0.00 / 500 / 30.0 1.00 / 100 / 0.00 / 500 / 30.0 5.00 / 0.00 / 100 / 500 / 30.0 6.00 / 0.00 / 100 / 500 / 30.0 6.50 / 100 / 0.00 / 500 / 30.0 17.0 / 100 / 0.00 / 500 / 30.0
Diverter Valve (Valco)	Time (min) / Position 0.00 / B
Injection Volume	5.00 µL
Total Run Time	20.0 minutes
Period 1-Experiment 1 **CSAG** **CSEG**	Scan Type/Polarity: MRM/Negative: GS1 = 50, GS2 = 70, CUR = 30.0, CAD = 9, IS = -4500, TEM = 500, EP = -10 Quantitation: (323/194 amu), DP = -35, CE = -26, CXP = -19 Confirmation: (323/238 amu), DP = -35, CE = -14, CXP = -27 Retention Time: Approximately 5.0 minutes Quantitation: (337/252 amu), DP = -120, CE = -22, CXP = -23 Confirmation: (337/77.9 amu), DP = -120 CE = -38, CXP = -9 Retention Time: Approximately 5.2 minutes