INTRODUCTION

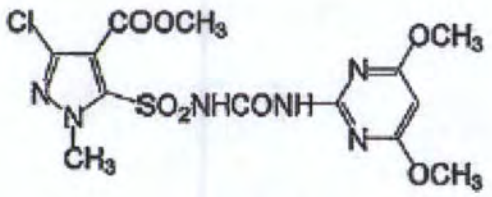
The purpose of this study was to develop and validate methods for the determination of halosulfuron-methyl (HSM) and its seven degradates, namely, halosulfuron-methyl rearrangement ester (RRE), 3-chlorosulfonamide acid methyl ester (CPSA or CSE) 2-amino-4,6-dimethoxypyrimidine (AP), 3-chlorosulfonamide acid (CSA), halosulfuron acid (HS), halosulfuron acid guanidine (CSAG) and chlorosulfonamide guanidine (CSEG) in both surface and sediment. The analysis of the test substances was performed by Liquid Chromatography with Tandem Mass Spectrometry Detection (LC-MS/MS) based on the methods developed in this study.

This study was designed to satisfy US EPA Guideline requirements described in OCSPP 850.6100. The study was initiated on October 28, 2014. The experimental work was conducted from November 11, 2014 through September 23, 2015 at PTRL West, 625-B Alfred Nobel Drive, Hercules, CA 94547 under an approved protocol (Appendix A) according to the US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160.

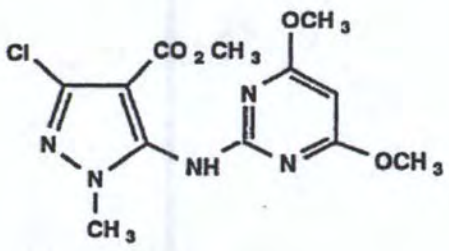
MATERIAL AND METHODS

Test and Reference Substances

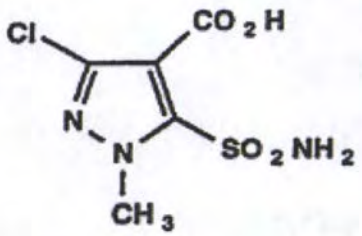
Name:	Halosulfuron-methyl (HSM)
IUPAC name:	methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate
Supplier:	Gowan Company
Lot No.:	110706
PTRL Inv. No.:	596W-355B
Storage Condition:	Room temperature
CAS No.:	100784-20-1
Molecular formula:	$C_{13}H_{15}ClN_6O_7S$
Molecular weight:	434.8 grams/mole
Purity:	99.0%
Expiration Date:	July 6, 2016
Structure:	



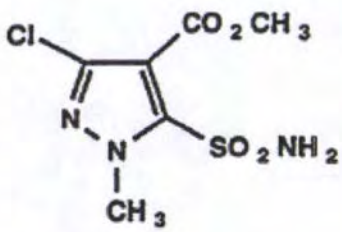
Name:	Halosulfuron-methyl rearrangement ester (RRE)
IUPAC name:	methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylamino)-1-methylpyrazole-4-carboxylate
Supplier:	Nissan Chemical Industries, Ltd.
Lot No.:	035-030618-1
PTRL Inv. No.:	596W-357
Storage Condition:	Freeze
Molecular formula:	$C_{12}H_{14}ClN_5O_4$
Molecular weight:	327.7 grams/mole
Purity:	100.0%
Expiration Date:	April 10, 2019
Structure:	



Name: 3-Chlorosulfonamide acid (CSA)
IUPAC name: 3-chloro-1-methyl-5-sulfamoylpyrazole-4-carboxylic acid
Supplier: Nissan Chemical Industries, Ltd.
Lot No.: CPSA-ACID-S9101
PTRL Inv. No.: 596W-356
Storage Condition: Freeze
Molecular formula: $C_5H_6ClN_3O_4S$
Molecular weight: 239.6 grams/mole
Purity: 99.9%
Expiration Date: June 21, 2016
Structure:

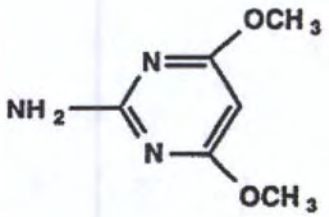


Name: 3-Chlorosulfonamide acid methyl ester (CPSA or CSE)
IUPAC name: Methyl 3-chloro-1-methyl-5-sulfamoylpyrazole-4-carboxylate
Supplier: Nissan Chemical Industries, Ltd.
Lot No.: CPSA-S931205
PTRL Inv. No.: 596W-382
Storage Condition: Refrigerate
CAS No.: 100784-27-8
Molecular formula: $C_6H_8ClN_3O_4S$
Molecular weight: 253.7 grams/mole
Purity: 99.8%
Expiration Date: August 25, 2019
Structure:

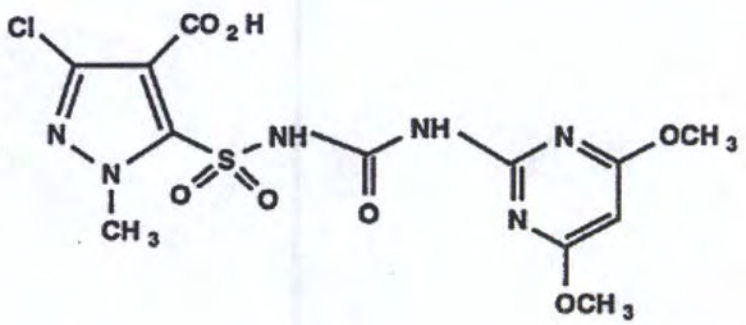


Name: 2-Amino-4,6-dimethoxypyrimidine (AP)

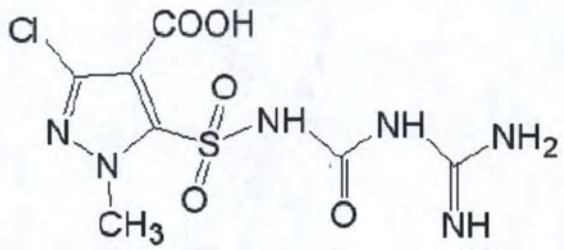
IUPAC name: 2-Amino-4,6-dimethoxypyrimidine
Supplier: Nissan Chemical Industries, Ltd.
Lot No.: SSDA309
PTRL Inv. No.: 596W-381
Storage Condition: Refrigerate
CAS No.: 36315-01-2
Molecular formula: $C_6H_9N_3O_2$
Molecular weight: 155.15 grams/mole
Purity: 100.0%
Expiration Date: Feb 26, 2019
Structure:



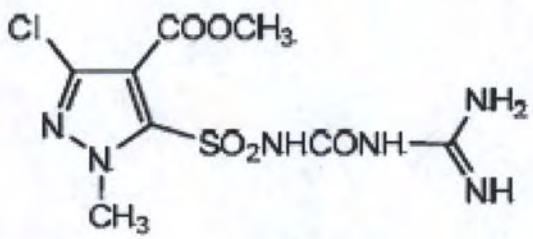
Name: Halosulfuron Acid (Halosulfuron or HS)
IUPAC name: 3- Chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoyl-sulfamoyl)-1-methlypyrazole-4-carboxylic acid
Supplier: Nissan Chemical Industries, Ltd.
Lot No.: 319ACID-S050331
PTRL Inv. No.: 2679W-001
Storage Condition: Refrigerate
CAS No.: 135397-30-7
Molecular formula: $C_{12}H_{13}ClN_6O_7S$
Molecular weight: 420.8 grams/mole
Purity: 99.9%
Expiration Date: October 29, 2015
Structure:



Name:	Halosulfuron acid guanidine or Chlorosulfonamide acid guanidine (CSA-guanidine, CSA-g or CSAG)
IUPAC name:	5-(carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylic acid
Supplier:	Nissan Chemical Industries, Ltd.
Lot No.:	CSAG-S091224
PTRL Inv. No.:	2679W-002
Storage Condition:	Refrigerate
Molecular formula:	$C_7H_9ClN_6O_5S$
Molecular weight:	324.7 grams/mole
Purity:	97.5%
Expiration Date:	May 7, 2019
Structure:	



Name:	Halosulfuron guanidine or Chlorosulfonamide guanidine (CSE-guanidine, CSE-g or CSEG)
IUPAC name:	methyl-5-(carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylate
Supplier:	Nissan Chemical Industries, Ltd.
Lot No.:	CSEG-S091224
PTRL Inv. No.:	2679W-003
Storage Condition:	Refrigerate
Molecular formula:	$C_8H_{11}ClN_6O_5S$
Molecular weight:	338.7 grams/mole
Purity:	92.9%
Expiration Date:	May 7, 2019
Structure:	



Certificates of Analysis for the reference and test substances are provided in Appendix B.

Other Chemicals

Acetonitrile, dichloromethane, ethyl acetate, and HPLC grade water were obtained from Burdick & Jackson; Sand was obtained from Acros Organics; celite was obtained from EMD Chemicals; acetic acid, formic acid, and hydrochloric acid were obtained from Fisher Scientific. Q100® QuEChERS salts (1g NaCl, 4g $MgSO_4$) were obtained from Restek.

Equipment List

Amber bottles and vials with Teflon® lined caps
Autosampler vials with snap caps and glass inserts
Beakers
Büchner funnels (6 cm & 12 cm diameter)
Disposable centrifuge tubes (50 mL capacity)
Glass concentration flasks (250 mL capacity)
Glass conical tubes (15 mL capacity)
Glass filter flasks (125 mL capacity)
Glass funnels (6 cm diameter)
Glass wool
Graduated glass cylinders
Hamilton glass precision syringes
IKA® RV8 rotovaps with water bath
Laboratory Balances
Microcentrifuge
Microcentrifuge filters, 0.45 µm Nylon
N-EVAP® 12-position nitrogen evaporator
Pasteur pipettes
Adjustable volume pipetors with plastic disposable tips
Separatory funnels (250 mL capacity)
SPEX ® GenoGrinder 2010 with 4mm SS grinder balls
Sorvall ® RT7 centrifuge
Thermometers
Volumetric flasks
Vortex mixer
Whatman ® qualitative filter paper, Grade 4 (5.5 cm & 11 cm dia.)

Whatman ® syringeless filters, 0.2 µm Nylon
Wrist-action shaker
AB Sciex API 5500 Series Triple Quad Mass Spectrometer with Thermo Scientific Agilent 1260 series Liquid Chromatograph (LC-MS/MS)

Test System

Source of Test System

The test systems are soil (PTRL Inventory No. 2439W-074) and sediment (PTRL Inventory No. 2706W-018). The soil/sediment samples were stored refrigerated (typically < 4°C) in the dark when not in use.

Characterization of the Test System

The soil/sediment samples used in the study were characterized by Agvise Laboratories, Inc. (604 Highway 15 West, Northwood, North Dakota) under PTRL West study 2439W or 2706W. The characterization reports are presented in Appendix C.

Test Method

The analytical methods for the analysis of eight compounds validated at PTRL West by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) were developed in this study.

Three residue methods were developed and validated: the first one for HSM/RRE/CPSA(CSE)/AP, the second one for CSA/HS, and the third one for CSAG/CSEG. For each method, the method validation experiment for soil and sediment matrix was conducted with one reagent blank, two untreated controls and five control samples spiked for each fortification level: one at LOQ level and another at 10X LOQ level. In HSM/RRE/CPSA(CSE)/AP method, target compounds were extracted from soil and sediment samples with acetonitrile and water, dichloromethane and ethyl acetate, followed by liquid partition and concentration steps with solvent evaporation. The final concentrated extract was reconstituted with acetonitrile/water and analyzed by LC-MS/MS. In CSA/HS method, target compounds were extracted from soil and sediment samples with 1% acetic acid in acetonitrile using principles of the QuEChERS method, followed by homogenization and centrifuge steps. The final concentrated extract was

directly analyzed by LC-MS/MS. In CSAG/CSEG method, target compounds were extracted from soil and sediment samples with acetonitrile, water, and concentrated HCl using principles of the QuEChERS method, followed by homogenization and centrifuge steps. The final concentrated extract was directly analyzed by LC-MS/MS.

Preparation of HSM Stock Solution

A stock solution of the HSM reference substance was prepared by weighing an aliquot (25.13 mg) of the reference substance (PTRL Inventory No. 596W-355B) onto a glass boat and then transferred into a 50 mL volumetric flask. The stock solution was dissolved and diluted with 50 mL of acetonitrile to yield a nominal concentration of 0.50 mg/mL after adjusted for the purity of the reference substance (99.0%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of RRE Stock Solution

A stock solution of the RRE reference substance was prepared by weighing an aliquot (25.06 mg) of the reference substance (PTRL Inventory No. 596W-357) onto a glass boat and then transferred into a 50 mL volumetric flask. The stock solution was dissolved and diluted with 50.12 mL of acetonitrile to yield a nominal concentration of 0.50 mg/mL after adjusted for the purity of the reference substance (100.0%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of CPSA(CSE) Stock Solution

A stock solution of the CPSA(CSE) reference substance was prepared by weighing an aliquot (25.13 mg) of the reference substance (PTRL Inventory No. 596W-382) onto a glass boat and then transferred into a 50 mL volumetric flask. The stock solution was dissolved and diluted with 50.159 mL of acetonitrile to yield a nominal concentration of 0.50 mg/mL after adjusted for the purity of the reference substance (99.8%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of AP Stock Solution

A stock solution of the AP reference substance was prepared by weighing an aliquot (25.08 mg) of the reference substance (PTRL Inventory No. 596W-381) onto a glass boat and then transferred to a 50 mL volumetric flask. The stock solution was dissolved and diluted with 50.16 mL of acetonitrile to yield a nominal concentration of 0.50 mg/mL after adjusted for the purity of the reference substance (100%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of CSA Stock Solution

A stock solution of the CSA reference substance was prepared by weighing an aliquot (25.15 mg) of the reference substance (PTRL Inventory No. 596W-356) onto a glass boat and then transferred into a 50 mL volumetric flask. The stock solution was dissolved and diluted with 50.25 mL of acetonitrile to yield a nominal concentration of 0.50 mg/mL after adjusted for the purity of the reference substance (99.9%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of HS Stock Solution

A stock solution of the HS reference substance was prepared by weighing an aliquot (5.16 mg) of the reference substance (PTRL Inventory No. 2679W-001) onto a glass boat and then transferred into a 50 mL volumetric flask. The stock solution was dissolved and diluted with 51.548 mL of acetonitrile to yield a nominal concentration of 0.10 mg/mL after adjusted for the purity of the reference substance (99.9%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of CSAG Stock Solution

A stock solution of the CSAG reference substance was prepared by weighing an aliquot (25.75 mg) of the reference substance (PTRL Inventory No. 2679W-002) onto a glass boat and then transferred into a 50 mL volumetric flask. The stock solution was dissolved and diluted with 50.213 mL of 1:1 (v:v) acetonitrile:HPLC-grade water to yield a nominal concentration of 0.50 mg/mL after adjusted for the purity of the reference

substance (97.5%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of CSEG Stock Solution

A stock solution of the CSEG reference substance was prepared by weighing an aliquot (27.52 mg) of the reference substance (Inventory No. 2679W-003) onto a glass boat and then transferred into a 50 mL volumetric flask. The stock solution was dissolved and diluted with 51.132 mL of 1:1 (v:v) acetonitrile:water to yield a nominal concentration of 0.50 mg/ after adjusted for the purity of the reference substance (92.9%). The stock solution was transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

Preparation of HSM and RRE Fortification Solution and Calibration Standard Solutions

The 250 ng/mL mixed fortification solution was prepared by measuring 0.05 mL of each stock solution into a 100 mL volumetric flask. Final solution was diluted to the mark with acetonitrile. This mixed stock solution contains 250 ng/mL of each compound. The fortification solution was mixed, transferred into three amber bottles and stored in the freezer (typically < -10°C) when not in use.

Six calibration standard solutions were prepared by measuring an appropriate volume of 250 ng/mL mixed fortification solution into 10 mL volumetric flasks via syringes as shown below. Final solutions were diluted to the mark with an appropriate volume of 1:1 (v:v) ACN:HPLC-grade water. The calibration solutions were mixed, transferred into amber vials and stored in the refrigerator (typically 2 to 6°C) when not in use.

Theoretical Conc. (ng/mL) Each compound	Volume of 250 ng/mL Solution (µL)	Final Volume (mL)
25	1000	10
10	400	10
5	200	10
2.5	100	10
1	40	10
0.5	20	10

To accommodate extract dilutions, three additional calibration solutions were prepared freshly for each analysis by mixing an appropriate volume of calibration standard solutions via adjustable volume pipetor with an appropriate volume of 1:1 (v:v)

ACN:HPLC-grade water directly into autosampler vials and vortexed to mix, for LC-MS/MS analysis. The standard solutions ranged from 0.05 ng/mL to 0.25 ng/mL can be prepared as shown below:

Theoretical Conc. (ng/mL) Each compound	Conc. of Soln. Used in Prep (ng/mL)	Volume of Soln Used in Prep (µL)	Volume of ACN:H_2O (µL)	Final Volume (mL)
0.25	0.5	100	100	0.2
0.1	1	100	900	1
0.05	0.5	100	900	1

Preparation of CPSA(CSE), CSEG and AP Fortification Solution and Calibration Standard Solutions

The following solutions were not used during the validation of CSEG due to solution instability.

The 500 ng/mL mixed fortification solution was prepared by measuring 0.1 mL of each stock solution into a 100 mL volumetric flask. Final solution was diluted to the mark with acetonitrile. This mixed stock solution contains 500 ng/mL of each compound. The fortification solution was mixed, transferred into three amber bottles and stored in the freezer (typically < -10°C) when not in use.

Six calibration standard solutions were prepared by measuring an appropriate volume of 500 ng/mL mixed fortification solution into 10 mL volumetric flasks via syringes as shown below. Final solutions were diluted to the mark with an appropriate volume of 1:1 (v:v) ACN:HPLC-grade water. The calibration solutions were mixed, transferred into amber vials and stored in the refrigerator (typically 2 to 6°C) when not in use.

Theoretical Conc. (ng/mL) Each compound	Volume of 500 ng/mL Solution (µL)	Final Volume (mL)
50	1000	10
20	400	10
10	200	10
5	100	10
2	40	10
1	20	10

To accommodate extract dilutions, three additional calibration solutions were prepared freshly for each analysis by mixing an appropriate volume of calibration standard

solutions via adjustable volume pipetor with an appropriate volume of 1:1 (v:v) ACN:HPLC-grade water directly into autosampler vials and vortexed to mix, for LC-MS/MS analysis. The standard solutions ranged from 0.1 ng/mL to 0.5 ng/mL can be prepared as shown below:

Theoretical Conc. (ng/mL) Each compound	Conc. of Soln. Used in Prep (ng/mL)	Volume of Soln Used in Prep (µL)	Volume of ACN:H_2O (µL)	Final Volume (mL)
0.5	1	100	100	0.2
0.2	2	100	900	1
0.1	1	100	900	1

Preparation of HS,CSA and CSAG Fortification Solution and Calibration Standard Solutions

The following solutions were not used during the validation of CSAG due to solution instability.

The 500 ng/mL mixed intermediate fortification solution was prepared by measuring 0.5 mL of HS stock solution, 0.1 mL of CSA stock solution and 0.1 mL of CSAG stock solution into a 100 mL volumetric flask. The final solution was diluted to the mark with acetonitrile. This mixed stock solution contains 500 ng/mL of each compound. The fortification solution was mixed, transferred into three amber bottles and stored in the freezer (typically < -10°C) when not in use.

Six calibration standard solutions were prepared by measuring an appropriate volume of 500 ng/mL mixed intermediate fortification solution into 10 mL volumetric flasks via syringes as shown below. Final solutions were diluted to the mark with an appropriate volume of 1:1 (v:v) ACN:HPLC-grade water. The calibration solutions were mixed, transferred into amber vials and stored in the refrigerator (typically 2 to 6°C) when not in use.

Theoretical Conc. (ng/mL) Each compound	Volume of 500 ng/mL Solution (µL)	Final Volume (mL)
50	1000	10
20	400	10
10	200	10
5	100	10
2	40	10
1	20	10

To accommodate extract dilutions, three additional calibration solutions were prepared freshly for each analysis by mixing an appropriate volume of calibration standard solutions via adjustable volume pipetor with an appropriate volume of 1:1 (v:v) ACN:HPLC-grade water directly into autosampler vials and vortexed to mix, for LC-MS/MS analysis. The standard solutions ranged from 0.1 ng/mL to 0.5 ng/mL can be prepared as shown below:

Theoretical Conc. (ng/mL) Each compound	Conc. of Soln. Used in Prep (ng/mL)	Volume of Soln Used in Prep (µL)	Volume of ACN:H_2O (µL)	Final Volume (mL)
0.5	1	100	100	0.2
0.2	2	100	900	1
0.1	1	100	900	1

Preparation of CSAG and CSEG Fortification Solution and Calibration Standard Solutions

The 1 µg/mL CSAG intermediate fortification solution was prepared by measuring 0.1 mL of CSAG stock solution into a 50 mL volumetric flask. Final solution was diluted to the mark with 1:1 (v:v) ACN:HPLC-grade water. This mixed stock solution contains 1 µg/mL of the compound. The fortification solution was vortexed to mix, transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

The 20 ng/mL CSAG fortification solution was prepared by measuring 2.0 mL of the 1 µg/mL CSAG intermediate fortification solution into a 100 mL volumetric flask. Final solution was diluted to the mark with 1:1 (v:v) ACN:HPLC-grade water. This mixed stock solution contains 20 ng/mL of the compound. The fortification solution was sonicated to mix, transferred into three amber bottles and stored in the refrigerator (typically 2 to 6°C) when not in use.

The 1 µg/mL CSEG intermediate fortification solution was prepared by measuring 0.1 mL of CSEG stock solution into a 50 mL volumetric flask. Final solution was diluted to the mark with 1:1 (v:v) ACN:HPLC-grade water. This mixed stock solution contains 1 µg/mL of the compound. The fortification solution was vortexed to mix, transferred into an amber bottle and stored in the freezer (typically < -10°C) when not in use.

The 20 ng/mL CSEG fortification solution was prepared by measuring 2.0 mL of the 1 µg/mL CSEG intermediate fortification solution into a 100 mL volumetric flask. Final solution was diluted to the mark with 1:1 (v:v) ACN:HPLC-grade water. This mixed

stock solution contains 20 ng/mL of the compound. The fortification solution was sonicated to mix, transferred into three amber bottles and stored in the refrigerator (typically 2 to 6°C) when not in use.

Due to the instability of CSAG and CSEG, it is recommended that the calibration solutions be freshly prepared for the analysis. Eight additional calibration standard solutions were prepared by mixing an appropriate volume of fortification or calibration solutions via adjustable volume pipetor with an appropriate volume of 1:1 (v:v) ACN:HPLC-grade water directly into autosampler vials and vortexed to mix, for LC-MS/MS analysis. The standard solutions ranged from 0.04 ng/mL to 10 ng/mL can be prepared as shown below:

Theoretical Conc. (ng/mL) Each compound	Conc. of Soln. Used in Prep (ng/mL)	Volume of Soln Used in Prep (µL)	Volume of ACN:H_2O (µL)	Final Volume (mL)
10	1000	20 of each	1960	2
5	10	500	500	1
2	10	200	800	1
1	10	100	900	1
0.5	5	100	900	1
0.2	2	100	900	1
0.1	1	100	900	1
0.01	0.2	50	950	1

Fortification Procedure

Fortification of untreated soil and sediment samples was conducted at two fortification levels as shown below:

	Fortification Level (ppb or ng/g)	Fortification Procedure
HSM & RRE:	0.5	0.020 mL of 250 ng/mL fort solution in 10 g of soil
	5	0.200 mL of 250 ng/mL fort solution in 10 g of soil

	Fortification Level (ppb or ng/g)	Fortification Procedure
CPSA(CSE) & AP:	1	0.020 mL of 500 ng/mL fort solution in 10 g of soil
	10	0.200 mL of 500 ng/mL fort solution in 10 g of soil

	Fortification Level (ppb or ng/g)	Fortification Procedure
HS & CSA:	1	0.020 mL of 500 ng/mL fort solution in 10 g of soil
	10	0.200 mL of 500 ng/mL fort solution in 10 g of soil

	Fortification Level (ppb or ng/g)	Fortification Procedure
CSAG & CSEG:	1	0.010 mL of 1 µg/mL intermediate fort solutions in 10 g of soil
	10	0.100 mL of 1 µg/mL intermediate fort solutions in 10 g of soil

Fortification was conducted to determine the percent recovery within the method validation. This procedure was performed in quintuplicate during method validation at each fortification level for each matrix.

Method for HSM/RRE/CPSA(CSE)/AP in Soil and Sediment

1. Weigh 10g of soil/sediment into a 50mL new plastic disposable centrifuge tube.
2. Add 2g of celite and 3.3g of sand to the sample.
3. Fortify as needed (LOQ - 0.5ppb for HSM/RRE and 1ppb for CPSA(CSE)/AP).
4. Add 20mL of ACN and 5mL deionized water. Place on a wrist-action shaker for 10 minutes at 50% speed.
5. Centrifuge sample at 3000 rpm for 5 minutes using the Sorvall RT-7 centrifuge.
6. Filter the supernatant through a Whatman No. 4 filter into a 125mL suction flask with the aid of a vacuum (water aspirator). The filter is supported in a Buchner funnel connected to the suction flask.
7. Break up soil plug in centrifuge tube and repeat steps 4-6.
8. Break up soil plug in centrifuge tube and add 20mL of dichloromethane and place on a wrist-action shaker for 10 minutes at 50% speed (setting 5). Repeat steps 5-6.
9. Repeat step 8.
10. Rinse the 50mL centrifuge tube and filter cake with 10mL dichloromethane.
11. Transfer the filtrate to a 250mL separatory funnel.
12. Rinse the 125mL suction flask with 30 mL of dichloromethane, sonicate and transfer to the 250mL separatory funnel containing the filtrate.
13. Shake the funnel vigorously by hand for 2 minutes. Allow the phases to separate for 5 minutes.
14. Drain the lower organic layer into a clean 250 mL concentration flask.
15. Add 30mL of ethyl acetate to the separatory funnel, and repeat step 13.

16. Drain lower aqueous layer into waste. Collect the upper organic layer in the same 250 mL concentration flask as step 14. Rinse the separatory funnel with 5 mL of ethyl acetate and add to concentration flask. Rinse the filter funnel with 10mL of ethyl acetate.
17. Rotary evaporate the combined extract to ~ 5 mL at 150 mbar, at 30 °C using the IKA RV-8 RotoVaps.
18. Filter through a 0.22 µm filter into a 15mL disposable glass tube. Rinse the flask with 5 mL of ethyl acetate and combine into the same 15 mL glass tube through the same filter.
19. Evaporate (N_2) to dryness, at 30 °C, using the NEVap system.
20. Reconstitute the sample in 2 mL of 1:1 (v:v) ACN:H_2O and vortex well.
21. Aliquot in LC vials for analysis by LC-MS/MS.

A schematic diagram of the method for HSM/RRE/CPSA(CSE)/AP in soil and sediment is presented in Figure 1.

Method for HS/CSA in Soil and Sediment

1. Weigh 10 g of soil/sediment into 50 ml disposable plastic centrifuge tubes.
2. Fortify as necessary (LOQ - 1 ng/g (ppb) for HS and CSA).
3. Add 10 mL of 1% acetic acid in acetonitrile, 5 mL H_2O, and (4) 4mm SS grinding balls to each sample.
4. Place on SPEX GenoGrinder at 1500 rpm for 2 minutes.
5. Add Restek Q100 unbuffered extraction salts (1 g NaCl, 4 g $MgSO_4$) to each tube.
6. Shake for 5 minutes on wrist-action shaker.
7. Centrifuge at 3000 rpm for 5 minutes using the Sorvall RT-7
8. Microfilter through a 0.45µm filter into amber vials for storage.
9. Aliquot to autosampler vial for analysis by LC-MS/MS.

A schematic diagram of the method for HS/CSA in soil and sediment is presented in Figure 2.

Method for CSAG and CSEG in Soil and Sediment

1. Weigh 10 grams of soil/sediment into 50 ml disposable plastic centrifuge tubes.
2. Fortify as necessary (LOQ - 1 ng/g for CSAG and CSEG).

3. Add 5 mL of 1% acetic acid in ACN, 5mL of HPLC H_2O and (4) 4mm SS grinding balls to each tube.
4. Shake for 2 minutes on SPEX GenoGrinder at 1500rpm, then centrifuge at 4000rpm for 10min using Sorvall RT-7.
5. Decant soil extract into a 50 mL graduated cylinder.
6. Break apart soil plug, then add 5 mL of 1% acetic acid in ACN and 5mL of HPLC H_2O to the centrifuge tube.
7. Shake for 2 minutes on SPEX GenoGrinder at 1500rpm, then centrifuge at 4000rpm for 5 min using Sorvall RT-7. Combine soil extracts in the same 50 mL graduated cylinder.
8. Rinse the 50mL centrifuge tube with 1:1 (v:v) ACN:HPLC H_2O and add rinse to the graduated cylinder, then adjust the final volume to 30 mL.
9. Transfer solution in each graduated cylinder to one new disposable plastic centrifuge tube. Add 1mL of concentrated HCl, Restek Q100 unbuffered extraction salts (1 g NaCl, 4 g $MgSO_4$), and (4) 4mm SS grinding balls to each tube.
10. Shake for 2 minutes on SPEX GenoGrinder at 1500rpm.
11. Centrifuge at 3000 rpm for 5 minutes using the Sorvall RT-7
12. Transfer solvent layer into amber vials for storage.
13. Filter extract through a 0.2µm nylon microcentrifuge filter prior to analysis.

A schematic diagram of the method for CSAG and CSEG in soil and sediment is presented in Figure 3.

Liquid Chromatography with Tandem Mass Spectrometry Analytical Method (LC-MS/MS)

LC-MS Parameters for HSM/RRE/CPSA(CSE)/AP

Column: Phenomenex Synergi ® 4µ Hydro-RP, 75x2.0mm, outfitted with a Phenomenex Security Guard ® Aqueous c18, 4x2mm guard cartridge (part no: AJO-7510)
Injection volume: 5 µL
Column oven temperature: 30 °C
Flow rate: 0.5 mL/min
Run time: 13 minutes
Mobile Phase:

- *A: 0.1% Formic acid in HPLC grade water*
- *B: 0.1% Formic acid in HPLC grade Acetonitrile*

Gradient Program:

Time (minutes)	%A	%B	Flow rate (mL/min)
0.0	100	0	0.5
1.0	100	0	0.5
5.0	0	100	0.5
9.0	0	100	0.5
9.5	100	0	0.5
13	100	0	0.5

ESI Positive mode, Experiment 1 (for AP)

MRM Parameters

Collision Gas (CAD)	9
Curtain Gas (CUR)	40
Gas 1 (GS1)	50
Gas 2 (GS2)	70
Temperature (TEM)	500
Exit Potential (EP)	10
MS1 resolution	unit
MS2 resolution	low

ESI Negative mode, Experiment 2 (for CPSA(CSE))

MRM Parameters

Collision Gas (CAD)	9
Curtain Gas (CUR)	40
Gas 1 (GS1)	50
Gas 2 (GS2)	70
Temperature (TEM)	500
Exit Potential (EP)	-10
MS1 resolution	unit
MS2 resolution	low

ESI Positive mode, Experiment 3 (for HSM and RRE)

MRM Parameters

Collision Gas (CAD)	6
Curtain Gas (CUR)	40
Gas 1 (GS1)	50
Gas 2 (GS2)	70
Temperature (TEM)	500
Exit Potential (EP)	10
MS1 resolution	unit
MS2 resolution	unit

Compound name	Precursor ion	Product ion	Collision Energy (CE)	Cell Exit Potential (CXP)	Declustering Potential (DP)	Dwell (msec)
HSM	434.9	182.2	27	16	35	40
	434.9	139.1	63	14	35	40
RRE	328.0	197.0	43	16	110	40
	328.0	295.9	19	24	110	40
AP	156.1	99.9	23	16	75	40
	156.1	57.0	30	12	75	40
CPSA(CSE)	252.0	187.9	-18	-15	-120	40
	252.0	219.8	-20	-19	-120	40

LC-MS Parameters for HS/CSA

Column: Phenomenex Synergi ® 4µ Hydro-RP, 75x2.0mm (Column #600), outfitted with a Phenomenex Security Guard ® Aqueous c18, 4x2mm guard cartridge (part no: AJO-7510)
Injection volume: 5 µL
Column oven temperature: 30 °C
Flow rate: 0.5 mL/min
Run time: 10 minutes
Mobile Phase:

- *A: 0.1% Formic acid in HPLC grade water*
- *B: 0.1% Formic acid in HPLC grade Acetonitrile*

Gradient Program:

Time (minutes)	%A	%B	Flow rate (mL/min)
0.0	100	0	0.5
1.0	100	0	0.5
5.0	0	100	0.5
6.0	0	100	0.5
6.5	100	0	0.5
10	100	0	0.5

ESI Negative mode

MRM Parameters

Collision Gas (CAD)	9
Curtain Gas (CUR)	40
Gas 1 (GS1)	50
Gas 2 (GS2)	70
Temperature (TEM)	500
Exit Potential (EP)	-10
MS1 resolution	unit
MS2 resolution	low

Compound name	Precursor ion	Product ion	Collision Energy (CE)	Cell Exit Potential (CXP)	Declustering Potential (DP)	Dwell (msec)
HS	419.0	238.0	-14	-25	-60	50
	419.0	194.0	-30	-15	-60	50
CSA	238.0	78.0	-31	-7.5	-40	50
	238.0	194.0	-16	-9	-40	50

LC-MS Parameters for CSAG/CSEG

Column: Phenomenex Synergi ® 4µ Hydro-RP, 75x2.0mm (Column #600-), outfitted with a Phenomenex Security Guard ® Aqueous c18, 4x2mm guard cartridge (part no: AJO-7510)
Injection volume: 5 µL
Column oven temperature: 30 °C
Flow rate: 0.5 mL/min
Run time: 10 minutes
Mobile Phase:

- *A: 0.1% Formic acid in HPLC grade water*
- *B: 0.1% Formic acid in HPLC grade Acetonitrile*

Gradient Program:

Time (minutes)	%A	%B	Flow rate (mL/min)
0.0	100	0	0.5
1.0	100	0	0.5
5.0	0	100	0.5
6.0	0	100	0.5
6.5	100	0	0.5
10	100	0	0.5

ESI Negative mode – MRM Parameters

Collision Gas (CAD)	9
Curtain Gas (CUR)	40
Gas 1 (GS1)	50
Gas 2 (GS2)	70
Temperature (TEM)	500
Exit Potential (EP)	-10
MS1 resolution	unit
MS2 resolution	low

Compound name	Precursor ion	Product ion	Collision Energy (CE)	Cell Exit Potential (CXP)	Declustering Potential (DP)	Dwell (msec)
CSAG	322.9	193.8	-26	-19	-35	50
	322.9	237.8	-14	-27	-35	50
CSEG	337.0	251.9	-22	-23	-120	50
	337.0	77.9	-38	-9	-120	50

LC-MS/MS Analysis

Samples were analyzed interspersed between the calibrants so as to assess the response of the calibrants if they had been affected by matrix samples (signal suppression or enhancement). Since separate linear curves were prepared for each compound, samples were interspersed between each calibration standards. Calibrants and samples were analyzed in single injection.

Methods of Calculation

Quantitation

Separation of HSM and its seven degradates was achieved by LC-MS/MS. The compounds were identified by the coincidence of their retention times with their respective reference standards and MS characteristics. The quantitation of HSM and its degradates was conducted by peak area of each compound relative to the theoretical concentration of the calibrants.

The content of HSM and its degradates in samples was quantified against separate 1/x weighted linear curves ($y = mx + b$) of calibrants where:

y = peak area
x = ng/mL compound injected
m = slope
b = intercept

Weighting of the calibration curve of each compound was applied so as to provide better curve fit at the lower concentration levels of each compound. The calculation of weighted

curve equations (linear regression) and concentration (ng/mL) present in samples and calibrants was conducted using Analyst® software.

Recoveries from fortified samples were determined by averaging the found concentration of each compound and dividing by the relevant fortification level.

Transcriptions (spreadsheets) of the raw data to support calculations in soil matrix are presented in Appendix D. Transcriptions (spreadsheets) of the raw data to support calculations in sediment matrix are presented in Appendix E.

Calibration Range

The calibration curve was generated by Analyst® software range from 0.05 ng/mL to 10 ng/mL for HSM/RRE, 0.1 ng/mL to 20 ng/mL for CPSA(CSE)/AP, 0.1 ng/mL to 50 ng/mL for HS/CSA, and 0.1 ng/mL to 10 ng/mL for CSAG/CSEG.

Limit of Quantitation

The limit of quantitation (LOQ) was validated at 0.5 ppb for HSM/RRE and 1.0 ppb for CPSA(CSE)/AP/CSA/HS/CSAG/CSEG.

Limit of Detection

The limit of detection (LOD) was demonstrated as the lowest calibrant concentration that gave a linear response and had a signal intensity consistently higher than that of reagent blank or control matrix responses. The LOD for each analyte is summarized as follows:

Analytes	LOD concentration (ng/mL)	LOD ppb equivalence (ng/g)	LOQ (ng/g)	% of LOQ
HSM/RRE	0.05	0.1	0.5	20%
CPSA/AP	0.1	0.2	1.0	20%
HS/CSA	0.1	0.1	1.0	10%
CSA-g/CSE-g	0.1	0.15	1.0	15%

Further discussion of LOD is provided in Results and Discussion section of this report.

Time Required for Completion of a Sample Set

For each method sample set consisted of a reagent blank, two controls (untreated soil and sediment samples) and five fortified soil and sediment samples at each fortification level. Time required for one sample set from initiation of extraction until the completion of instrumental analysis and data evaluation is as follows:

- Sample preparation takes approximately 8 hours for HSM/RRE/CPSA(CSE)/AP method
- LC-MS/MS analysis and data processing (two MS/MS transitions for each compound) take approximately 6 hours for HSM/RRE/CPSA(CSE)/AP method
- Sample preparation takes approximately 4 hours for CSA/HS method
- LC-MS/MS analysis and data processing (two MS/MS transitions for each compound) take approximately 4 hours for CSA/HS method
- Sample preparation takes approximately 6 hours for CSAE/CSEG method
- LC-MS/MS analysis and data processing (two MS/MS transitions for each compound) take approximately 4 hours for CSAE/CSEG method

TOTAL = approximately 32 hours for one analyst to complete sample sets for three methods (approximately four calendar days) to satisfy the validation requirements of each matrix evaluated.

Statistical Methods

Means, standard deviation, relative standard deviation, and 1/x linear regression were the only statistical methods employed in this study.

Figure 1. Schematic Diagram of the Analytical Procedure for HSM/RRM/CPSA(CSE)/AP.

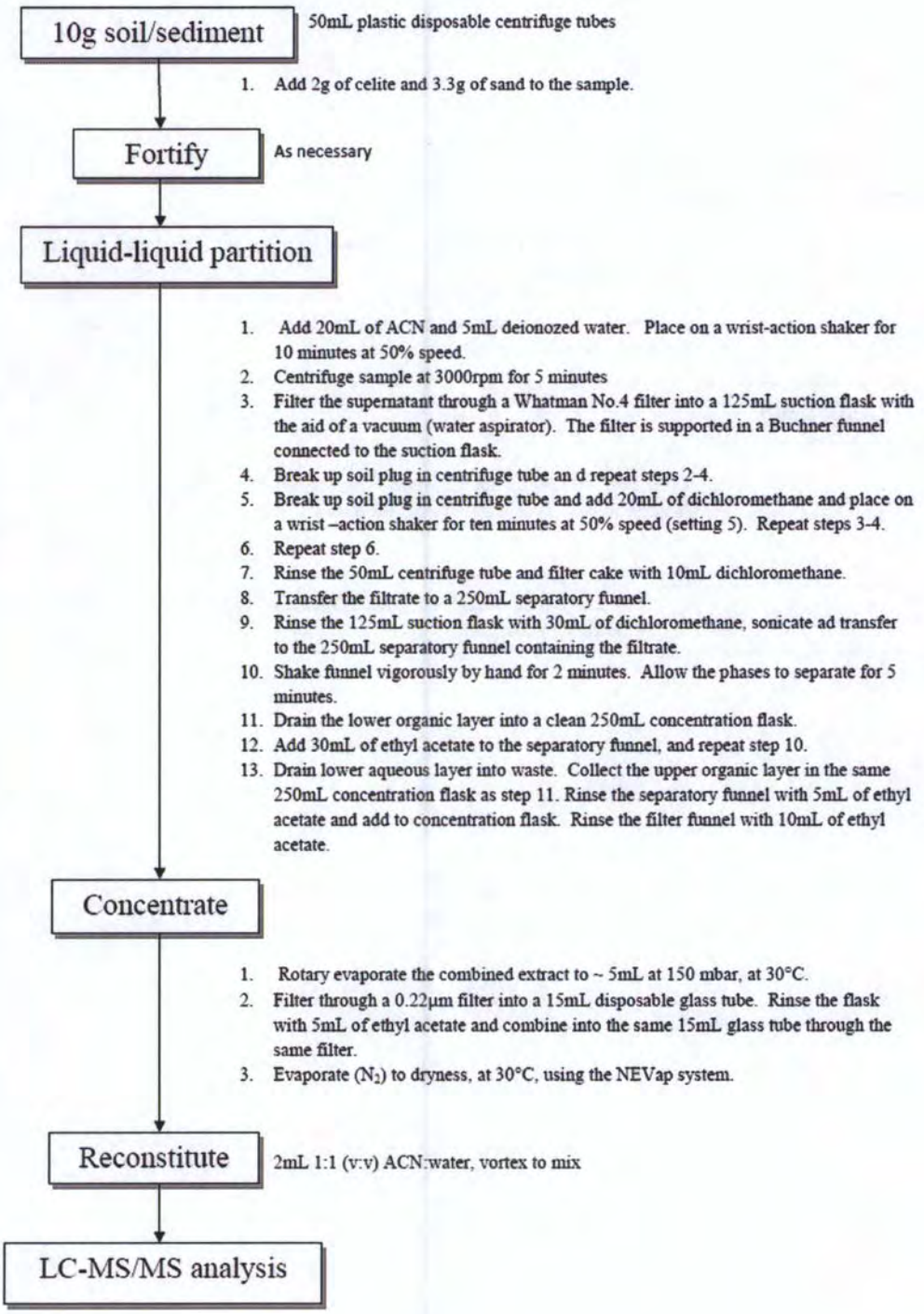


Figure 2. Schematic Diagram of the Analytical Procedure for HS/CSA.

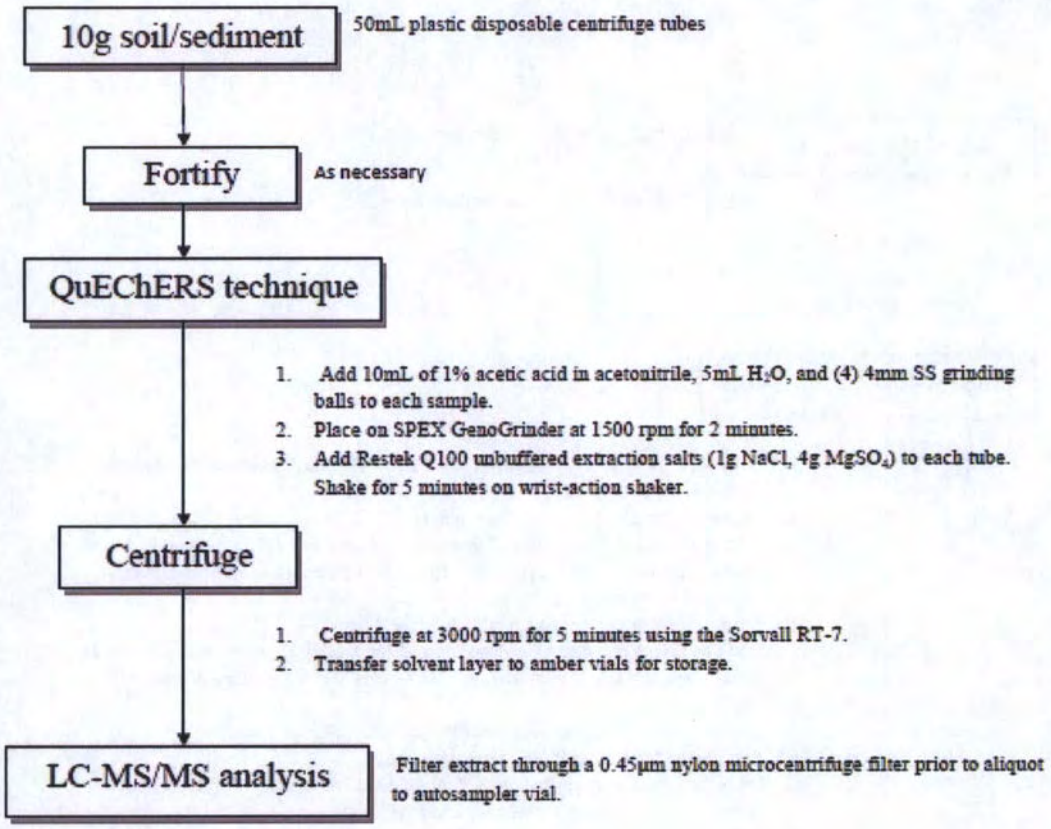


Figure 3. Schematic Diagram of the Analytical Procedure for CSAG /CSEG.