Analytical method for halosulfuron-methyl (HSM) and its transformation products halosulfuron-methyl rearrangement ester (RRE), 3-chlorosulfonamide acid methyl ester (CPSA or CSE), 2-amino-4,6-dimethoxypyrimidine (AP), halosulfuron acid (HS), 3-chlorosulfonamide (CSA), halosulfuron acid guanidine (CSAG) and halosulfuron ester guanidine (CSEG) in soil and sediment

Reports: ECM: EPA MRID No.: 49798402. Shen, H. and T. Arndt. 2015. Development and Validation of a Method for the Determination of Halosulfuron-methyl (HSM) and its Degradates in Soil/Sediment. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California, sponsored and submitted by Gowan Company, Yuma, Arizona; 204 pages. PTRL Study No: 2678W. Final report issued December 7, 2015.

ILV: EPA MRID No.: 49983102. MacGregor, J.A. and E.S. Bodle. 2016. INDEPENDENT LABORATORY VALIDATION OF METHODS FOR THE DETERMINATION OF HALOSULFURON-METHYL (HSM) AND ITS DEGRADATES IN SOIL/SEDIMENT BY LC/MS/MS. Report prepared by EAG Laboratories, Easton, Maryland, sponsored and submitted by Canyon Group LLC, Yuma, Arizona and Gowan Company, Yuma, Arizona; 196 pages. EAG Laboratories Project No: 334C-132. Final report issued July 27, 2016.

Document No.: MRIDs 49798402 & 49983102

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49798402). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the QA statement.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (p. 3 of MRID 49983102). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was not included.

Classification: This analytical method is classified as supplemental. The soil and sediment matrices of the ILV were the same as those used in the ECM. In the ILV, method recoveries of CSAG in soil matrix at the LOQ and 10×LOQ did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy for both ions. In the ECM, the method RSD for HSM in soil matrix at the LOQ for the quantification ion did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy for both ions. In the ECM, method recoveries at the LOQ and 10×LOQ of AP in sediment matrix and of CSAG in soil matrix did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy. In the ILV, linearity was not satisfactory for CSEG. The representative chromatograms of ILV analysis of RRE in soil did not support the specificity of the method. In the ECM, the LOQ chromatograms for HSM, CPSA, AP, CSA and CSAG in soil or soil/sediment matrices showed baseline interferences with peak integration. The LODs for the analytes were not reported in the ILV.

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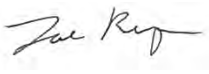
EFED Final Reviewers:	Zoe Ruge, Physical Scientist	Signature:	
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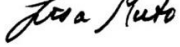
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature:	
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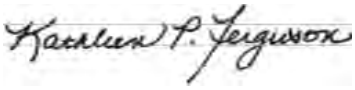
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature:	
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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The analytical method, PTRL Study No: 2678W, is designed for the quantitative determination of halosulfuron-methyl (HSM) and its transformation products halosulfuron-methyl rearrangement ester (RRE), 3-chlorosulfonamide acid methyl ester (CPSA or CSE), 2-amino-4,6-dimethoxypyrimidine (AP), halosulfuron acid (HS), 3-chlorosulfonamide (CSA), halosulfuron acid guanidine (CSAG) and halosulfuron ester guanidine (CSEG) in soil/sediment using HPLC/MS/MS. In soil/sediment, the method is quantitative for halosulfuron-methyl and RRE at the stated LOQ of 0.5 ppb and for CPSA, AP, HS, CSA, CSAG and CSEG at the LOQ of 1.0 ppb. The acceptability of the LOQs cannot be determined because the lowest toxicological level of concern in soil is currently unknown. At this time, LOD/LOQ should be within the values specified in the terrestrial field study guidance.[1] Characterized sandy clay loam soil and sand sediment were used for the ECM validation (USDA soil textural classification); the specific sources of the soil/sediment matrices were not reported. The soil and sediment matrices of the ILV were the same as those used in the ECM. The ECM method for HSM/RRE/CPSA (CSE)/AP, CSA/HS and CSAG/CSEG was validated by the ILV for both soil and sediment matrices in the first trial with insignificant modifications to the sample processing procedure. All ILV data regarding repeatability, accuracy, and precision were satisfactory for all analytes in both matrices, except for CSAG in soil matrix. In the ILV, linearity was not satisfactory for CSEG. All ILV data regarding specificity were satisfactory for all analytes in both matrices, except for RRE in soil; only quantitation ion chromatograms were provided for all analytes. The LODs for the analytes were not reported in the ILV. All ECM data regarding repeatability, accuracy, and precision were satisfactory for all analytes in both matrices, except for HSM and CSAG in soil and AP in sediment. All ECM data regarding specificity were satisfactory for all analytes in both matrices, except that baseline interferences affected the integration of the LOQ peaks for HSM (confirmation ion only), CPSA, AP and CSA in soil and CSAG in soil and sediment.

[1] URL: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/nafta-guidance-document-conducting-terrestrial-field

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Halosulfuron-methyl (HSM)	49798402[2]	49983102[3]	Supplemental	Soil and Sediment	7/12/15	Gowan Company, LLC Canyon Group LLC	LC/MS/MS	0.5 ppb
RRE								
CPSA (CSE)								1.0 ppb
AP								
CSA								
HS								
CSAG								
CSEG								

1 HSM = Methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate. RRE = Halosulfuron-methyl rearrangement ester; Methyl 3-chloro-5-[(4,6-dimethoxypyrimidin-2-yl)amino]-1-methyl-pyrazole-4-carboxylate. CPSA/CSE = 3-Chlorosulfonamide acid methyl ester; Methyl-3-chloro-1-methyl-5-sulfamoylpyrazole-4-carboxylate. AP = Aminopyrimidine; 2-Amino-4,6-dimethoxypyrimidine. HS = Halosulfuron acid; 3- Chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methlypyrazole-4-carboxylic acid. CSA = 3-Chlorosulfonamide; 3-Chloro-1-methyl-5-sulfamoyl-pyrazole-4-carboxylic acid. CSAG = Halosulfuron acid guanidine; 5-(Carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylic acid. CSEG = Halosulfuron ester guanidine; Methyl 5-(carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylate.

2 Characterized sandy clay loam soil (57% sand, 22% silt, 21% clay; organic matter 3.1%) and sand sediment (91% sand, 5% silt, 4% clay; organic matter 2.2%) were used for the ECM validation (USDA soil textural classification; p. 22; Appendix C, pp. 185-186 of MRID 49798402). The specific sources of the soil/sediment matrices were not reported.

3 In the ILV, sandy clay loam soil (2439W-074; 57% sand, 22% silt, 21% clay; organic matter 3.1%; pH 6.2) and sand sediment (2706W-018; 91% sand, 5% silt, 4% clay; organic matter 2.2%; pH 5.8) were provided by PTRL West (the ECM laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil textural classification; p. 14; Appendix III, pp. 176-177 of MRID 49983102). The specific sources of the soil/sediment matrices were not reported. The matrices were the same as those used in the ECM.

I. Principle of the Method

Extraction procedure for HSM/RRE/CPSA (CSE)/AP: Soil/sediment (10 g) with celite (2 g) and sand (3.3 g) in a 50-mL disposable plastic centrifuge tubes was fortified with 0.02 or 0.20 mL of 250 ng/mL HSM and RRE fortification solutions or 0.02 or 0.20 mL of 500 ng/mL CPSA (CSE) and AP fortification solutions (pp. 22-23, 29-31; Figure 1, p. 53 of MRID 49798402). Acetonitrile (20 mL) and deionized water (5 mL) were mixed with the soil sample via shaking on a wrist-action shaker for 10 minutes at 50% speed. After centrifugation (5 minutes at 3000 rpm) using the Sorvall RT-7, the supernatant was filtered through a Buchner filter funnel containing a Whatman No. 4 filter into a 125-mL suction flask using vacuum (water aspirator). The filter was rinsed with 10 mL of dichloromethane. The remaining soil pellet was broken up, and the extraction was repeated. The soil extracts were combined. After the second extraction, the remaining soil pellet was broken up, and the extraction was repeated twice with dichloromethane (20 mL) in the same manner as before. The dichloromethane extracts were combined with the acetonitrile/water extracts. The centrifuge tube and filter cake were rinsed with 10 mL of dichloromethane. The filtrate was transferred to a 250-mL separatory funnel. The 125-mL suction flask was rinsed with 30 mL of dichloromethane. After sonication, the rinsate was transferred to the 250-mL separatory funnel. The funnel was shaken vigorously by hand for 2 minutes. After 5 minutes to allow the phases to separate, the lower layer was drained. The aqueous phase was extracted with 30 mL of ethyl acetate in the same manner as before. The lower aqueous layer was drained to waste. The ethyl acetate layer was combined with the dichloromethane extract. The separatory funnel was rinsed with 5 mL of ethyl acetate; the filter funnel was rinsed with 10 mL of ethyl acetate. The combined extracts and rinses were reduced to *ca*. 5 mL via rotary evaporation at 150 mbar and 30°C using the IKA RV-8 RotoVaps. The residue was filtered (0.22 µm filter) and transferred to a 15-mL disposable glass tube. The flask was rinsed with 5 mL of ethyl acetate which was combined with the residue in the disposable glass tube. The solvent was evaporated under a gentle stream of nitrogen to dryness at 30°C. The residue was reconstituted in 2 mL of acetonitrile:water (1:1, v:v) and mixed via vortex prior to LC/MS/MS analysis.

Extraction procedure for CSA/HS: Soil/sediment (10 g) in a 50-mL disposable plastic centrifuge tubes was fortified with 0.02 or 0.20 mL of 500 ng/mL HS and CSA fortification solutions (pp. 22-23, 30-31; Figure 2, p. 54 of MRID 49798402). The soil/sediment was extracted with 1% acetic acid in acetonitrile (10 mL) and water (5 mL) with 4 4-mm SS grinding balls via shaking for 2 minutes on SPEX GenoGrinder at 1500 rpm. Restek Q100 unbuffered extraction salts (1 g NaCl, 4 g $MgSO_4$) were added then the sample was shaken for 5 minutes on a wrist-action shaker. After centrifugation (5 minutes at 3000 rpm) using the Sorvall RT-7, the supernatant was filtered (0.45 µm filter) and transferred to amber vials. An aliquot was transferred to an autosampler vial for LC/MS/MS analysis.

Extraction procedure for CSAG/CSEG: Soil/sediment (10 g) in a 50-mL disposable plastic centrifuge tubes was fortified with 0.10 or 1.00 mL of 1 µg/mL CSAG and CSEG fortification solutions (pp. 22-23, 30, 31-32; Figure 3, p. 55 of MRID 49798402). The soil/sediment was extracted twice with 1% acetic acid in acetonitrile (5 mL) and HPLC water (5 mL) with 4 4-mm SS grinding balls via shaking for 2 minutes on SPEX GenoGrinder at 1500 rpm. After centrifugation (10 minutes at 4000 rpm) using the Sorvall RT-7, the supernatant was transferred to a 50-mL graduated cylinder. The centrifuge tube was rinsed with acetonitrile:HPLC water (1:1, v:v; volume not reported). The rinse was combined with the extracts, and the volume was adjusted to 30 mL. The rinse and extracts were transferred to a fresh disposable plastic centrifuge tube and mixed with

concentrated HCl (1 mL) and Restek Q100 unbuffered extraction salts (1 g NaCl, 4 g $MgSO_4$) with 4 4-mm SS grinding balls via shaking for 2 minutes on SPEX GenoGrinder at 1500 rpm. After centrifugation (5 minutes at 3000 rpm) using the Sorvall RT-7, the supernatant was transferred to amber vials. An aliquot was filtered (0.2 µm nylon microcentrifuge filter) and transferred to an autosampler vial for LC/MS/MS analysis.

LC/MS/MS: Samples are analyzed using an AB Sciex API 5500 Series Triple Quad Mass Spectrometer with Thermo Scientific Agilent 1260 series Liquid Chromatograph (p. 22). The following LC conditions were used (pp. 33-36 of MRID 49798402): Phenomenex Synergi® 4µ Hydro-RP column (2.0 mm x 75 mm, column temperature 30°C), Phenomenex Security Guard® Aqueous c18 guard column (4 mm x 2 mm), mobile phase of (A) 0.1% formic acid in HPLC grade water and (B) 0.1% formic acid in HPLC grade acetonitrile, and injection volume of 5 µL. LC mobile phase gradient and MS multiple reaction monitoring (MRM) conditions are reported below based on analyte.

HSM/RRE/CPSA (CSE)/AP: The following mobile phase gradient was used (pp. 33-34 of MRID 49798402): percent A:B (v:v) at 0.0-1.0 min. 100:0, 5.0-9.0 min. 0:100, 9.5-13 min. 100:0. The MRM parameters were ESI positive mode for AP (Experiment 1), ESI negative mode for CPSA (CSE; Experiment 2), and ESI positive mode for HSM and RRE (Experiment 3). Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 434.9→182.2 and *m/z* 434.9→139.1 for HSM, *m/z* 328.0→295.9 and *m/z* 328.0→197.0 for RRE, *m/z* 156.1→99.9 and *m/z* 156.1→57.0 for AP, and *m/z* 252.0→187.9 and *m/z* 252.0→219.8 for CPSA (CSE). Expected retention times were 5.28, 4.99, 3.51, and 4.37 minutes for HSM, RRE, AP, and CPSA (CSE), respectively (Figure 11, pp. 112-115).

CSA/HS: The following mobile phase gradient was used (pp. 34-35 of MRID 49798402): percent A:B (v:v) at 0.0-1.0 min. 100:0, 5.0-6.0 min. 0:100, 6.5-10 min. 100:0. The MRM parameters were ESI negative mode. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 419.0→194.0 and *m/z* 419.0→238.0 for HS, and *m/z* 238.0→78.0 and *m/z* 238.0→194.0 for CSA. Expected retention times were 3.69 and 4.70 minutes for CSA and HS, respectively (Figure 11, pp. 116-117).

CSAG/CSEG: The following mobile phase gradient was used (pp. 36-37 of MRID 49798402): percent A:B (v:v) at 0.0-1.0 min. 100:0, 5.0-6.0 min. 0:100, 6.5-10 min. 100:0. The MRM parameters were ESI negative mode. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 322.9→193.8 and *m/z* 322.9→237.8 for CSAG, and *m/z* 337.0→251.9 and *m/z* 337.0→77.9 for CSEG. Expected retention times were 3.56 and 3.78 minutes for CSAG and CSEG, respectively (Figure 11, pp. 118-119).

ILV: The ILV performed the ECM methods for each analyte as written, except for insignificant equipment and procedure modifications (pp. 20-23; Tables 1-3, pp. 31-33 of MRID 49983102). The LC/MS/MS instrument and parameters were the same as those of the ECM. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 435→182 and *m/z* 435→139 for HSM, *m/z* 328→296 and *m/z* 328→197 for RRE, *m/z* 156→100 and *m/z* 156→57 for AP, *m/z* 252→188 and *m/z* 252→220 for CPSA (CSE), *m/z* 419→194 and *m/z* 419→238 for HS, *m/z* 238→78.0 and *m/z* 238→194 for CSA, *m/z* 323→194 and *m/z* 323→238 for CSAG, and *m/z* 337→252 and *m/z* 337→77.9 for CSEG (see Reviewer's Comment #10; Tables 1-3, pp. 31-33;

Figures 4-27, pp. 69-92). Expected retention times were *ca*. 6.4, 6.3, 5.0, 5.8, 5.0, 5.8, 5.0 and 5.2 minutes for HSM, RRE, AP, CPSA (CSE), CSA, HS, CSAG and CSEG, respectively.

The following critical step was noted by the ILV: in the method for HSM/RRE/CPSA (CSE)/AP, care must be taken to minimize the length of time sample extracts are allowed to remain at dryness when on the nitrogen evaporator system (Appendix V, pp. 194-195 of MRID 49983102). The ILV study authors also noted the general issue that the HPLC gradient profile was modified to include a longer mobile phase gradient equilibration period to prevent the observed chromatographic issues associated with the early eluting peaks.

LOQ and LOD: In the ECM and ILV, the Limits of Quantification (LOQ) were 0.5 ppb for HSM and RRE and 1.0 ppb for CPSA (CSE), AP, HS, CSA, CSAG and CSEG (p. 10 of MRID 49798402; pp. 12-13, 20 of MRID 49983102). In the ECM, the Limits of Detection (LOD) were 0.1 ppb for HSM and RRE, 0.2 ppb for CPSA (CSE) and AP, 0.1 ppb for HS and CSA, and 0.15 ppb for CSAG and CSEG. The LODs for the analytes were not reported in the ILV.

II. Recovery Findings

ECM (MRID 49798402): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of halosulfuron-methyl (HSM) and its transformation product RRE at fortification levels of 0.5 ppb (LOQ) and 5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 1.0 ppb (LOQ) and 10 ppb (10×LOQ) in the soil matrix, except for RSDs of HSM which were 24-26% at the LOQ (ions combined) and mean recoveries of CSAG which were 59-65% at the LOQ and 10×LOQ (ions combined; Tables I-II, pp. 46-50). Mean recoveries and RSDs were within guidelines for analysis of halosulfuron-methyl (HSM) and its transformation product RRE at fortification levels of 0.5 ppb (LOQ) and 5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 1.0 ppb (LOQ) and 10 ppb (10×LOQ) in the sediment matrix, except for the mean recoveries of AP which were 53-62% at the LOQ and 10×LOQ (ions combined). Two ion pair transitions were monitored for each analyte using LC/MS/MS in either positive or negative ESI mode. The quantification and confirmation ion data was comparable or fairly comparable for all analytes in both matrices. Sandy clay loam soil (2439W-074; 57% sand, 22% silt, 21% clay; organic matter 3.1%; pH 6.2) and sand sediment (2706W-018; 91% sand, 5% silt, 4% clay; organic matter 2.2%; pH 5.8) were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil textural classification; p. 22; Appendix C, pp. 185-186). The specific sources of the soil/sediment matrices were not reported.

ILV (MRID 49983102): Mean recoveries and RSDs were within guidelines for analysis of halosulfuron-methyl (HSM) and its transformation product RRE at fortification levels of 0.5 ppb (LOQ) and 5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 1.0 ppb (LOQ) and 10 ppb (10×LOQ) in the soil matrix, except for the mean recovery of CSAG at the LOQ and 10×LOQ which was 61.3-62.5% (ions combined; Tables 4-35, pp. 34-65). Mean recoveries and RSDs were within guidelines for analysis of halosulfuron-methyl (HSM) and its transformation product RRE at fortification levels of 0.5 ppb (LOQ) and 5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 1.0 ppb (LOQ) and 10 ppb (10×LOQ) in the sediment matrix. Two ion pair transitions were monitored for each analyte using LC/MS/MS in either positive or negative ESI mode. The quantification and confirmation ion data

was comparable or fairly comparable for all analytes in both matrices, except for CSA (LOQ only) in the soil matrix. Sandy clay loam soil (2439W-074; 57% sand, 22% silt, 21% clay; organic matter 3.1%; pH 6.2) and sand sediment (2706W-018; 91% sand, 5% silt, 4% clay; organic matter 2.2%; pH 5.8) were provided by PTRL West (the ECM laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil textural classification; p. 14; Appendix III, pp. 176-177). The specific sources of the soil/sediment matrices were not reported. The matrices were the same as those used in the ECM. The method was validated with insignificant modifications to the sample processing procedure (pp. 20-23; Tables 1-3, pp. 31-33). The method for HSM/RRE/CPSA (CSE)/AP, CSA/HS and CSAG/CSEG was validated in the first trial with soil and sediment matrices (Appendix V, pp. 195-196).

Table 2. Initial Validation Method Recoveries for Halosulfuron-methyl (HSM) and Its Transformation Products RRE, CPSA, AP, HS, CSA, CPAG and CPEG in Soil/Sediment[1]

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Clay Loam Soil[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5[4]	84.0-152	107	28	**26**
	5	5	74.6-84.8	81	4	5
RRE	0.5 (LOQ)	5	80.8-97.6	85	7	8
	5	5	73.4-84.0	80	4	5
AP	1.0 (LOQ)	5	67.8-79.0	76	5	6
	10	5	68.8-83.2	78	6	8
CPSA (CSE)	1.0 (LOQ)	5	79.0-108	100	12	12
	10	5	75.6-97.6	88	8	10
HS	1.0 (LOQ)	5	91.3-99.8	96	3	4
	10	5	93.2-101	98	3	4
CSA	1.0 (LOQ)	5	88.7-96.8	93	3	3
	10	5	85.0-89.1	88	2	2
CSAG	1.0 (LOQ)	5	47.1-65.6	**59**	8	14
	10	5	59.1-63.6	**61**	2	3
CSEG	1.0 (LOQ)	5	75.2-78.5	77	1	2
	10	5	73.8-81.8	78	3	4
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5[4]	84.4-146	107	26	**24**
	5	5	78.4-86.4	82	3	4
RRE	0.5 (LOQ)	5	82.4-102	88	8	9
	5	5	74.0-86.4	81	5	6
AP	1.0 (LOQ)	5	66.4-75.8	72	4	5
	10	5	65.2-78.8	73	5	7
CPSA (CSE)	1.0 (LOQ)	5	90.0-105	97	6	6
	10	5	83.6-101.0	93	6	7
HS	1.0 (LOQ)	5	98.8-110	103	5	4
	10	5	99.0-103	100	2	2
CSA	1.0 (LOQ)	5	79.0-89.9	85	4	5
	10	5	85.9-87.6	87	1	1

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
CSAG	1.0 (LOQ)	5	61.8-70.5	**65**	3	5
	10	5	59.0-66.9	**62**	3	5
CSEG	1.0 (LOQ)	5	79.4-84.6	83	2	3
	10	5	72.3-83.9	80	5	6
	Sand Sediment[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5	86.4-115	95	12	12
	5	5	64.6-88.0	75	10	14
RRE	0.5 (LOQ)	5	74.0-83.2	79	4	5
	5	5	63.6-81.6	74	7	10
AP	1.0 (LOQ)	5	52.8-70.0	**62**	8	12
	10	5	38.0-59.6	**53**	10	19
CPSA (CSE)	1.0 (LOQ)	5	80.2-106	93	10	11
	10	5	72.4-104	89	14	15
HS	1.0 (LOQ)	5	87.6-93.9	91	3	3
	10	5	86.8-91.5	90	2	2
CSA	1.0 (LOQ)	5	86.2-93.1	90	3	3
	10	5	86.0-89.3	87	2	3
CSAG	1.0 (LOQ)	5	81.6-91.1	87	4	5
	10	5	78.9-80.0	79	0	1
CSEG	1.0 (LOQ)	5	75.2-78.5	77	1	2
	10	5	73.8-81.8	78	3	4
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5	81.6-106	87	11	12
	5	5	62.4-90.4	77	13	17
RRE	0.5 (LOQ)	5	69.6-80.4	76	4	5
	5	5	61.2-80.0	73	8	11
AP	1.0 (LOQ)	5	55.2-68.8	**62**	5	9
	10	5	39.6-60.8	**53**	10	18
CPSA (CSE)	1.0 (LOQ)	5	80.2-97.4	88	7	8
	10	5	73.2-97.2	86	11	13
HS	1.0 (LOQ)	5	87.0-91.6	90	2	2
	10	5	85.4-90.1	87	2	2
CSA	1.0 (LOQ)	5	78.1-93.7	85	7	8
	10	5	86.4-93.9	89	3	4
CSAG	1.0 (LOQ)	5	77.6-86.3	82	3	4
	10	5	82.2-86.9	84	2	2
CSEG	1.0 (LOQ)	5	79.4-84.6	83	2	3

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	10	5	72.3-83.9	80	5	6

Data (uncorrected recovery results; pp. 37-38) were obtained from Tables I-II, pp. 46-50 of MRID 49798402.
Red values indicate discrepancies with meeting guideline requirements.

1 HSM = Methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate. RRE = Halosulfuron-methyl rearrangement ester; Methyl 3-chloro-5-[(4,6-dimethoxypyrimidin-2-yl)amino]-1-methyl-pyrazole-4-carboxylate. CPSA/CSE = 3-Chlorosulfonamide acid methyl ester; Methyl-3-chloro-1-methyl-5-sulfamoylpyrazole-4-carboxylate. AP = Aminopyrimidine; 2-Amino-4,6-dimethoxypyrimidine. HS = Halosulfuron acid; 3- Chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methlypyrazole-4-carboxylic acid. CSA = 3-Chlorosulfonamide; 3-Chloro-1-methyl-5-sulfamoyl-pyrazole-4-carboxylic acid. CSAG = Halosulfuron acid guanidine; 5-(Carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylic acid. CSEG = Halosulfuron ester guanidine; Methyl 5-(carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylate.

2 Sandy clay loam soil (2439W-074; 57% sand, 22% silt, 21% clay; organic matter 3.1%; pH 6.2) and sand sediment (2706W-018; 91% sand, 5% silt, 4% clay; organic matter 2.2%; pH 5.8) were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil textural classification; p. 22; Appendix C, pp. 185-186). The specific sources of the soil/sediment matrices were not reported.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 434.9→182.2 and *m/z* 434.9→139.1 for HSM, *m/z* 328.0→295.9 and *m/z* 328.0→197.0 for RRE, *m/z* 156.1→99.9 and *m/z* 156.1→57.0 for AP, *m/z* 252.0→187.9 and *m/z* 252.0→219.8 for CPSA (CSE), *m/z* 419.0→194.0 and *m/z* 419.0→238.0 for HS, *m/z* 238.0→78.0 and *m/z* 238.0→194.0 for CSA, *m/z* 322.9→193.8 and *m/z* 322.9→237.8 for CSAG, and *m/z* 337.0→251.9 and *m/z* 337.0→77.9 for CSEG.

4 For HSM in soil at the LOQ, one result was omitted by the study authors as an outlier for statistical analysis in the quantification and confirmation data set (n = 4; Table I, p. 46). No calculations, such as the Dixon test, were provided to support omitting the replicate. Means and RSDs for n =4 were 95% and 14%, respectively, for the quantification ion and 96% and 17%, respectively, for the confirmation ion. The statistical results presented in the table above were reviewer-calculated based on n = 5 (see DER Attachment 2).

Table 3. Independent Validation Method Recoveries for Halosulfuron-methyl (HSM) and Its Transformation Products RRE, CPSA, AP, HS, CSA, CPAG and CPEG in Soil/Sediment

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Clay Loam Soil[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5	90.5-103	96.1	6.33	**6.59**
	5	5	91.7-103	95.7	4.39	4.59
RRE	0.5 (LOQ)	5	95.6-123	108	10.7	9.91
	5	5	85.9-99.9	94.6	5.18	5.48
AP	1.0 (LOQ)	5	68.7-89.8	78.9	8.57	10.9
	10	5	74.3-87.2	82.5	4.86	5.89
CPSA (CSE)	1.0 (LOQ)	5	94.5-116	103	9.44	9.17
	10	5	89.2-102	97.1	4.81	4.95
HS	1.0 (LOQ)	5	90.8-106	101	5.90	5.84
	10	5	94.5-107	102	5.17	5.07
CSA	1.0 (LOQ)	5	89.0-94.1	91.4	2.12	2.32
	10	5	83.8-86.1	85.2	0.891	1.05
CSAG	1.0 (LOQ)	5	59.5-66.2	**61.3**	2.77	4.52
	10	5	61.6-62.9	**62.0**	0.515	0.831
CSEG	1.0 (LOQ)	5	97.5-104	101	3.04	3.01
	10	5	97.5-105	101	2.73	2.70
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5	81.8-100	93.7	9.31	9.94
	5	5	91.4-101	97.3	3.81	3.92
RRE	0.5 (LOQ)	5	97.0-125	110	10.3	9.36
	5	5	88.4-102	95.8	4.90	5.11
AP	1.0 (LOQ)	5	74.6-95.6	84.2	7.62	9.05
	10	5	74.5-87.0	83.2	5.07	6.09
CPSA (CSE)	1.0 (LOQ)	5	89.8-112	98.0	8.98	9.16
	10	5	88.5-108	96.3	8.14	8.45
HS	1.0 (LOQ)	5	92.8-106	99.9	5.18	5.19
	10	5	95.7-106	102	4.26	4.18
CSA	1.0 (LOQ)	5	72.0-82.9	78.3	4.26	5.44
	10	5	83.1-87.6	84.7	1.77	2.09
CSAG	1.0 (LOQ)	5	58.0-66.1	**62.1**	3.24	5.22
	10	5	61.5-63.6	**62.5**	0.935	1.50
CSEG	1.0 (LOQ)	5	101-107	103	2.61	2.53
	10	5	93.8-100	98.1	2.67	2.72
	Sand Sediment[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5	83.3-91.6	86.2	3.40	3.94
	5	5	88.7-93.3	90.7	1.65	1.82
RRE	0.5 (LOQ)	5	94.5-101	97.7	3.05	3.12
	5	5	93.0-98.7	96.0	2.21	2.30
AP	1.0 (LOQ)	5	72.3-84.8	81.0	5.04	6.22
	10	5	86.7-91.6	88.8	1.98	2.23
CPSA (CSE)	1.0 (LOQ)	5	95.8-113	102	6.85	6.72

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	10	5	97.2-109	104	6.13	5.89
HS	1.0 (LOQ)	5	89.6-100	97.0	4.49	4.63
	10	5	93.5-99.9	97.5	2.87	2.94
CSA	1.0 (LOQ)	5	81.1-86.4	84.7	2.17	2.56
	10	5	81.3-83.0	82.5	0.682	0.827
CSAG	1.0 (LOQ)	5	75.2-90.5	84.1	5.84	6.94
	10	5	83.3-86.9	84.5	1.45	1.72
CSEG	1.0 (LOQ)	5	107-116	113	4.09	3.62
	10	5	101-113	106	4.58	4.32
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.5 (LOQ)	5	73.6-92.8	85.0	7.24	8.52
	5	5	87.8-91.0	88.9	1.34	1.51
RRE	0.5 (LOQ)	5	91.2-97.6	95.8	2.59	2.70
	5	5	95.3-98.3	97.0	1.31	1.35
AP	1.0 (LOQ)	5	70.8-88.8	81.0	6.85	9.46
	10	5	87.4-90.9	89.2	1.48	1.66
CPSA (CSE)	1.0 (LOQ)	5	82.9-109	94.8	9.75	10.3
	10	5	98.9-115	106	6.41	6.05
HS	1.0 (LOQ)	5	91.2-98.1	96.2	2.95	3.07
	10	5	94.3-103	99.1	3.29	3.32
CSA	1.0 (LOQ)	5	73.0-83.7	78.1	4.09	5.24
	10	5	79.7-85.9	83.4	2.61	3.13
CSAG	1.0 (LOQ)	5	82.0-96.7	88.9	5.31	5.97
	10	5	83.0-84.5	84.0	0.926	1.11
CSEG	1.0 (LOQ)	5	109-117	112	3.27	2.92
	10	5	98.6-107	104	4.09	3.93

Data (uncorrected recovery results; pp. 23-25) were obtained from Tables 4-35, pp. 34-65 of MRID 49983102.
Red values indicate discrepancies with meeting guideline requirements.

1 HSM = Methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate. RRE = Halosulfuron-methyl rearrangement ester; Methyl 3-chloro-5-[(4,6-dimethoxypyrimidin-2-yl)amino]-1-methyl-pyrazole-4-carboxylate. CPSA/CSE = 3-Chlorosulfonamide acid methyl ester; Methyl-3-chloro-1-methyl-5-sulfamoylpyrazole-4-carboxylate. AP = Aminopyrimidine; 2-Amino-4,6-dimethoxypyrimidine. HS = Halosulfuron acid; 3- Chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methlypyrazole-4-carboxylic acid. CSA = 3-Chlorosulfonamide; 3-Chloro-1-methyl-5-sulfamoyl-pyrazole-4-carboxylic acid. CSAG = Halosulfuron acid guanidine; 5-(Carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylic acid. CSEG = Halosulfuron ester guanidine; Methyl 5-(carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylate.

2 Sandy clay loam soil (2439W-074; 57% sand, 22% silt, 21% clay; organic matter 3.1%; pH 6.2) and sand sediment (2706W-018; 91% sand, 5% silt, 4% clay; organic matter 2.2%; pH 5.8) were provided by PTRL West (the ECM laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil textural classification; p. 14; Appendix III, pp. 176-177). The specific sources of the soil/sediment matrices were not reported. The matrices were the same as those used in the ECM.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 435→182 and *m/z* 435→139 for HSM, *m/z* 328→296 and *m/z* 328→197 for RRE, *m/z* 156→100 and *m/z* 156→57 for AP, *m/z* 252→188 and *m/z* 252→220 for CPSA (CSE), *m/z* 419→194 and *m/z* 419→238 for HS, *m/z* 238→78.0 and *m/z* 238→194 for CSA, *m/z* 323→194 and *m/z* 323→238 for CSAG, and *m/z* 337→252 and *m/z* 337→77.9 for CSEG (see Reviewer's Comment #10).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD were 0.5 ppb for HSM and RRE and 1.0 ppb for CPSA (CSE), AP, HS, CSA, CSAG and CSEG (pp. 10, 38, 43-44 of MRID 49798402; pp. 12-13, 20; Tables 4-35, pp. 34-65 of MRID 49983102). In the ECM, the LOQs were defined by their validation in the study. In the ILV, the LOQs were defined as the lowest level fortified during the method validation set. No calculations or further justification was provided. In the ECM, the Limits of Detection (LOD) were 0.1 ppb for HSM and RRE, 0.2 ppb for CPSA (CSE) and AP, 0.1 ppb for HS and CSA, and 0.15 ppb for CSAG and CSEG. In the ECM, the LOD was defined as the lowest calibrant concentration that gave a linear response and had a signal intensity above that of the reagent blank or control matrix responses. The study authors also reported that the LOD was 20% or lower for all analytes. The LODs were 0.05 ng/mL for HSM and RRE and 0.1 ng/mL for all other analytes. The LOD ppb equivalence was calculated using the following equation:

LOD (ppb equivalence) = [LOD conc. (ng/mL) x final volume (mL) x Dilution Factor] ÷ sample weight (g).

No calculations of the LOD based on standard deviations or background levels were reported in the ECM. The LODs for the analytes were not reported in the ILV.

Table 4. Method Characteristics for Halosulfuron-methyl (HSM) and Its Transformation Products RRE, CPSA, AP, HS, CSA, CPAG and CPEG[1] in Soil/Sediment

			Halosulfuron-methyl HSM	RRE	CPSA (CSE)	AP	HS	CSA	CSAG	CSEG
Limit of Quantitation (LOQ)	ECM		0.5 ppb		1.0 ppb					
	ILV									
Limit of Detection (LOD)	ECM		0.1 ppb		0.2 ppb		0.1 ppb		0.15 ppb	
	ILV		Not reported							
Linearity (calibration curve r^2 and concentration range)	ECM[2]		r^2 = 0.9971 (Q) r^2 = 0.9987 (C)	r^2 = 0.9964 (Q) r^2 = 0.9990 (C)	r^2 = 0.9975 (Q) r^2 = 0.9978 (C)	r^2 = 0.9984 (Q) r^2 = 0.9996 (C)	r^2 = 0.9998 (Q) r^2 = 0.9995 (C)	r^2 = 0.9999 (Q) r^2 = 0.9999 (C)	r^2 = 0.9997 (Q) r^2 = 0.9994 (C)	r^2 = 0.9999 (Q) r^2 = 0.9989 (C)
		Range:	0.05-10 ng/mL		0.1-20 ng/mL		0.1-50 ng/mL		0.1-10 ng/mL	
	ILV[3]		r^2 = 0.9995 (Q)	r^2 = 0.9996 (Q)	r^2 = 0.9990 (Q)	r^2 = 0.9978 (Q)	r^2 = 0.9977 (Q)	r^2 = 0.9998 (Q)	r^2 = 0.9998 (Q)	r^2 = **0.9947** (Q)
		Range:	0.05-25 ng/mL		0.1-50 ng/mL		0.04-50 ng/mL		0.04-10 ng/mL	
Repeatable	ECM[4]	Soil:	Yes at 10×LOQ. **No** at LOQ, RSD = **24-26**% (Q).	Yes at LOQ and 10×LOQ.					**No**; mean recoveries **59-65**% at LOQ and **61-62**% at 10×LOQ.	Yes at LOQ and 10×LOQ.
		Sediment:	Yes at LOQ and 10×LOQ.			**No**; mean recoveries **62**% at LOQ and **53**% at 10×LOQ.	Yes at LOQ and 10×LOQ.			
	ILV[5]	Soil:	Yes at LOQ and 10×LOQ.						**No**; mean recoveries **61.3-62.1**% at LOQ and **62.0-62.5**% at 10×LOQ.	Yes at LOQ and 10×LOQ.
		Sediment:	Yes at LOQ and 10×LOQ.							
Reproducible			Yes at LOQ and 10×LOQ in soil and sediment matrices.						Yes at LOQ and 10×LOQ in sediment matrix; **No** at LOQ and 10×LOQ in soil	Yes at LOQ and 10×LOQ in soil and sediment matrices.

			Halosulfuron-methyl HSM	RRE	CPSA (CSE)	AP	HS	CSA	CSAG	CSEG
									matrix.	
Specific	ECM	Soil:	Interferences were 10-20% of LOQ, based on peak height, at analyte retention times; however, baseline noise interfered with analyte peak (C) integration.[6]	Interferences were <10% of LOQ, based on peak height, at analyte retention times.	No matrix interferences were observed; however, baseline noise interfered with analyte peak integration at the LOQ.[7]	No matrix interferences were observed; however, baseline noise interfered with analyte peak integration at the LOQ.[8]	No matrix interferences were observed.	A nearby peak from a contaminant and baseline noise interfered with analyte peak integration at the LOQ.[9]	Baseline noise interfered with analyte peak integration at the LOQ.[10]	Interferences were <10% of LOQ, based on peak height, at analyte retention times.
		Sediment:	Interferences were <10% of LOQ, based on peak height, at analyte retention times.		No matrix interferences were observed.			No matrix interferences were observed.	A nearby peak from a contaminant and baseline noise interfered with analyte peak integration at the LOQ.[11]	
	ILV	Only quantitation ion chromatograms were provided.								
		Soil:	No matrix interferences were observed.	Interferences were *ca*. 32-43% of LOQ, based on measured amounts.[12]	No matrix interferences were observed; however, baseline noise was significant near the analyte peak.[13]	No matrix interferences were observed.			No matrix interferences were observed; however, minor baseline noise was observed near the analyte peak.	No matrix interferences were observed.
		Sediment:		No matrix interferences were observed.					No matrix interferences were observed.	

Data were obtained from pp. 10, 12-15, 38, 43-44; Tables I-II, pp. 46-50; Figures 5-6, pp. 64-79 (reagent blanks and control soil chromatograms); Figure 7, pp. 80-87 (Calibration curves); Figures 11-12, pp. 112-127 (LOQ and 10×LOQ chromatograms for soil); Figures 13-15, pp. 128-151 (control sediment chromatograms and LOQ and 10×LOQ chromatograms for sediment); Figure 16, pp. 152-159 (LOD chromatograms) of MRID 49798402; pp. 12, 20; Tables 4-35, pp. 34-65 (recovery results); Figures 1-3, pp. 66-68 (calibration curves); Figures 4-27, pp. 69-92 (chromatograms) of MRID 49983102. Q = quantitation ion; C = confirmation ion. All results

reported for Q and C ions unless specified otherwise.
Red values indicate discrepancies with meeting guideline requirements.
1 HSM = Methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate. RRE = Halosulfuron-methyl rearrangement ester; Methyl 3-chloro-5-[(4,6-dimethoxypyrimidin-2-yl)amino]-1-methyl-pyrazole-4-carboxylate. CPSA/CSE = 3-Chlorosulfonamide acid methyl ester; Methyl-3-chloro-1-methyl-5-sulfamoylpyrazole-4-carboxylate. AP = Aminopyrimidine; 2-Amino-4,6-dimethoxypyrimidine. HS = Halosulfuron acid; 3- Chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methlypyrazole-4-carboxylic acid. CSA = 3-Chlorosulfonamide; 3-Chloro-1-methyl-5-sulfamoyl-pyrazole-4-carboxylic acid. CSAG = Halosulfuron acid guanidine; 5-(Carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylic acid. CSEG = Halosulfuron ester guanidine; Methyl 5-(carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylate.
2 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (pp. 25-29; Figure 8, pp. 88-95 of MRID 49798402; DER Attachment 2).
3 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; only one set of calibration cures was provided (Figures 1-3, pp. 66-68 of MRID 49983102; DER Attachment 2). The reviewer assumed that these curves were for the quantitation ion. The calibration curves were titled with "S". Calibration standards were prepared in solvent (pp. 17-19).
4 In the ECM, sandy clay loam soil (2439W-074; 57% sand, 22% silt, 21% clay; organic matter 3.1%; pH 6.2) and sand sediment (2706W-018; 91% sand, 5% silt, 4% clay; organic matter 2.2%; pH 5.8) were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil textural classification; p. 22; Appendix C, pp. 185-186 of MRID 49798402). The specific sources of the soil/sediment matrices were not reported.
5 In the ILV, sandy clay loam soil (2439W-074; 57% sand, 22% silt, 21% clay; organic matter 3.1%; pH 6.2) and sand sediment (2706W-018; 91% sand, 5% silt, 4% clay; organic matter 2.2%; pH 5.8) were provided by PTRL West (the ECM laboratory) and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil textural classification; p. 14; Appendix III, pp. 176-177 of MRID 49983102). The specific sources of the soil/sediment matrices were not reported. The matrices were the same as those used in the ECM.
6 Matrix and baseline interferences for the LOQ were most notable for the confirmation ion chromatogram (Figure 6, p. 72; Figure 11, p. 112 of MRID 49798402).
7 Figure 11, p. 114 of MRID 49798402.
8 Figure 11, p. 115; Figure 14, p. 139 of MRID 49798402.
9 Figure 11, p. 116 of MRID 49798402.
10 Figure 11, p. 118 of MRID 49798402.
11 Figure 14, p. 142 of MRID 49798402.
12 Tables 6-7, pp. 36-37 of MRID 49983102.
13 Figures 11-12, pp. 76-77 of MRID 49983102.
Linearity is satisfactory when $r^2 \geq 0.995$.
A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. ECM MRID 49798402 was originally submitted without an ILV. The ECM was reviewed without the ILV by CDM Smith primary reviewer Lisa Muto and secondary reviewer Kathleen Ferguson. The data from the ILV was combined with data from the previous DER. The DER content regarding the ECM MRID 49798402 was reviewed and adjusted, if necessary, based on data form the ILV, but, generally, very little modification was done to the original DER content regarding ECM MRID 49798402.

2. In the ILV, the soil and sediment matrices were the same as those used in the ECM (p. 22; Appendix C, pp. 185-186 of MRID 49798402; p. 14; Appendix III, pp. 176-177 of MRID 49983102). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

3. In the ILV, the analysis of CSAG did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%) in the soil matrix at the stated LOQ mean recoveries: 61.3% quantitation ion, 62.1% confirmation ion) and 10×LOQ (mean recoveries: 62.0% quantitation ion, 62.5% confirmation ion; Tables 16-17, pp. 46-47 of MRID 49983102). CSAG was identified as a major transformation product in an aerobic soil metabolism study (MRID 49031306); the reviewed method does not meet the guideline criteria for analyzing this degradate.

4. In the ECM, several compounds did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%) at the stated LOQ and at higher concentrations in both soil/sediment matrices. In the soil matrix, the RSDs of HSM were 24-26% at the LOQ (quantification and confirmation ions) and mean recoveries of CSAG were 59-65% at the LOQ and 10×LOQ (quantification and confirmation ions; Tables I-II, pp. 46-50 of MRID 49798402). In the sediment matrix, the mean recoveries of AP were 53-62% at the LOQ and 10×LOQ (quantification and confirmation ions). CSAG was identified as a major transformation product and AP was identified as a minor transformation product in an aerobic soil metabolism study (MRID 49031306); the reviewed method does not meet the guideline criteria for analyzing these degradates.

   For HSM in soil at the LOQ, one result was omitted by the study authors as an outlier for statistical analysis in the quantification and confirmation data set (n = 4; Table I, p. 46 of MRID 49798402). No calculations, such as the Dixon test, were provided to support omitting the replicate. Means and RSDs for n =4 (reported by the study authors) were 95% and 14%, respectively, for the quantification ion and 96% and 17%, respectively, for the confirmation ion. These results satisfy OCSPP Guideline 850.6100 criteria for precision and accuracy. The statistical results presented in the DER were reviewer-calculated based on n = 5 (see DER Attachment 2).

5. In the ILV, linearity was not satisfactory for CSEG (r^2 = 0.9947; pp. 17-19; Figure 3, p. 68 of MRID 49983102). Linearity is satisfactory when $r^2 \geq 0.995$. Additionally, only one set of calibration curves was provided. The reviewer assumed that these curves were for the quantitation ion. The calibration curves were titled with "S". The calibration standards were prepared in solvent. Since data for the confirmatory ion was reported in the ILV study

report, the corresponding calibration curves used to generate that data should have been reported. However, the reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

6. For the ILV analysis of RRE in soil, matrix interferences were *ca*. 32-43% of LOQ, based on measured amounts (Tables 6-7, pp. 36-37 of MRID 49983102). These matrix interferences were significant. The ILV study authors considered these interferences "to be isolated to the matrix blanks only as evident by fortification recoveries and by the absence of other analyte peaks" (footnotes; Tables 6-7, pp. 36-37). The ILV study authors also reported that there were no detectable peak above 30% of the LOQ in the reagent blank.

7. In the ILV, only quantitation ion chromatograms were provided; no chromatograms from the confirmatory ion analyses were shown (Figures 4-27, pp. 69-92 of MRID 49983102). The reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

   Also, baseline noise was significant near the analyte peak in ILV chromatograms of CPSA (Figures 11-12, pp. 76-77 of MRID 49983102).

8. In the ECM, LOQ chromatograms for CPSA (CSE), AP and CSAG in the soil matrix showed baseline interferences with peak integration (Figure 11, pp. 114-115, 118 of MRID 49798402). Baseline interference was also noted in the confirmation chromatograms for the LOQ of HSM in soil (Figure 11, p. 112). The reviewer noted that a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data. The LOQ chromatograms for CSA in soil and CSAG in sediment showed interference with peak integration due to a nearby peak of a contaminant, as well as baseline noise (Figure 11, p. 116; Figure 14, p. 142).

9. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ and LOD were not adequately supported by calculations or comparison to background levels in the ECM (pp. 10, 38, 43-44 of MRID 49798402; pp. 12-13, 20; Tables 4-35, pp. 34-65 of MRID 49983102). In the ECM, the LOQs were defined by their validation in the study. In the ILV, the LOQs were defined as the lowest level fortified during the method validation set. In the ECM, the LOD was defined as the lowest calibrant concentration that gave a linear response and had a signal intensity above that of the reagent blank or control matrix responses. The study authors also reported that the LOD was 20% or lower for all analytes. The LODs for the analytes were not reported in the ILV.

10. In the ILV, the reviewer noted several significant typographical errors in the reported monitored ion pair transitions for HSM, AP and CPSA in Table 1 (Table 1, p. 31; Figures 4-27, pp. 69-92 of MRID 49983102). Ion transitions for HSM were incorrectly reported as *m/z* 156→100 and *m/z* 156→57 in Table 1 (those for AP), instead of *m/z* 435→182 and *m/z* 435→139. Ion transitions for AP and CPSA were interchanged in Table 1.

11. In the ECM, matrix effects were evaluated in all matrices (p. 43; Table IV, p. 52 of MRID 49798402). The study authors determined that matrix effects (≥20%) were observed for HSM (-27.11%, soil), RRE (-55.11%, soil; -44.07%, sediment), AP (-32.50%, soil; -29.33%,

sediment), and CPSA (-24.74%, soil). The study authors did not use matrix-matched standards since they determined that the matrix effects could be reduced by diluting the final extracts with solvent by a factor of 10 prior to analysis.

12. The communications between the ILV and study developers and sponsors were detailed; communications involved discussions of trial successes (Appendix V, pp. 195-196 of MRID 49983102).

13. In the ILV, the total time required to perform the method (extraction and analysis) for all analytes with one sample set was *ca*. 7 days (Appendix V, p. 195 of MRID 49983102). One set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) required *ca*. 12 hours (processing) and *ca*. 12 hours (analysis and data processing) for the HSM/RRE/CPSA(CSE)/AP method, and *ca*. 4 hours (processing) and *ca*. 11 hours (analysis and data processing) for the HS/CSA method or CSAG/CSEG method.

    The total time required to perform the ECM (extraction and analysis) was *ca*. 32 hours (p. 39 of MRID 49798402). One set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) required *ca*. 8 hours (processing) and *ca*. 6 hours (analysis and data processing) for the HSM/RRE/CPSA(CSE)/AP method, *ca*. 4 hours (processing) and *ca*. 4 hours (analysis and data processing) for the HS/CSA method, and *ca*. 6 hours (processing) and *ca*. 4 hours (analysis and data processing) for the CSAG/CSEG method.

14. The ECM should be edited to account for the following critical step noted by the ILV: in the method for HSM/RRE/CPSA (CSE)/AP, care must be taken to minimize the length of time sample extracts are allowed to remain at dryness when on the nitrogen evaporator system (Appendix V, pp. 194-195 of MRID 49983102). The ILV study authors also noted the general issue that the HPLC gradient profile was modified to include a longer mobile phase gradient equilibration period to prevent the observed chromatographic issues associated with the early eluting peaks.

V. References

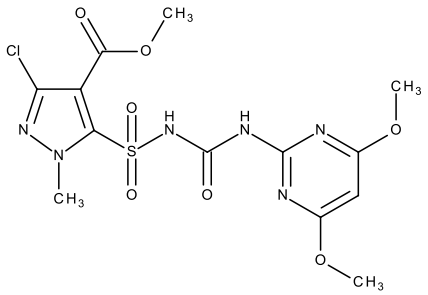
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

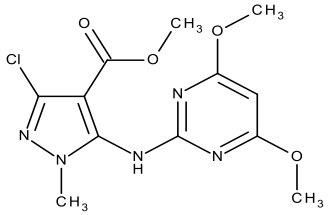
Halosulfuron-methyl (HSM; NC-319)

IUPAC Name:	Methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate
CAS Name:	Methyl 3-chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-1-methyl-1H-pyrazole-4-carboxylate
CAS Number:	100784-20-1
SMILES String:	COC(=O)c1c(Cl)nn(C)c1S(=O)(=O)NC(=O)Nc2nc(OC)cc(OC)n2



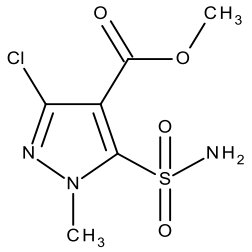
Halosulfuron-methyl rearrangement ester (RRE; HSMR)

IUPAC Name:	Methyl 3-chloro-5-[(4,6-dimethoxypyrimidin-2-yl)amino]-1-methyl-pyrazole-4-carboxylate
CAS Name:	Not reported
CAS Number:	Not found
SMILES String:	Cn1c(c(c(n1)Cl)C(=O)OC)Nc2nc(cc(n2)OC)OC



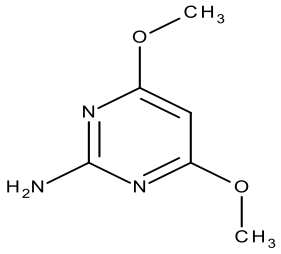
3-Chlorosulfonamide acid methyl ester (CPSA or CSE; Chlorosulfonamide)

IUPAC Name: Methyl-3-chloro-1-methyl-5-sulfamoylpyrazole-4-carboxylate
CAS Name: Not reported
CAS Number: 100784-27-8
SMILES String: Cn1c(c(c(n1)Cl)C(=O)OC)S(=O)(=O)N



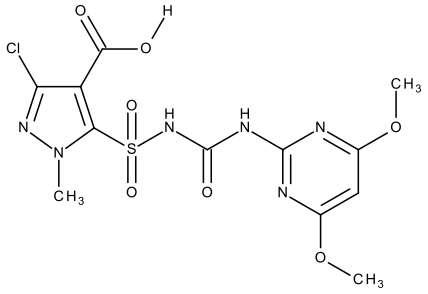
2-Amino-4,6-dimethoxypyrimidine (AP; ADMP; Aminopyrimidine; 620Pd-1)

IUPAC Name: 2-Amino-4,6-dimethoxypyrimidine
CAS Name: Not reported
CAS Number: 36315-01-2
SMILES String: COc1cc(nc(n1)N)OC



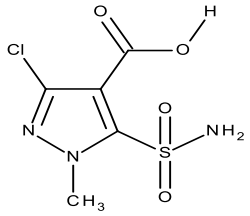
Halosulfuron acid (HS; Halosulfuron; 319-ACID; NC-319 ACID)

IUPAC Name: 3- Chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methlypyrazole-4-carboxylic acid
CAS Name: Not reported
CAS Number: 135397-30-7
SMILES String: Cn1c(c(c(n1)Cl)C(=O)O)S(=O)(=O)NC(=O)Nc2nc(cc(n2)OC)OC



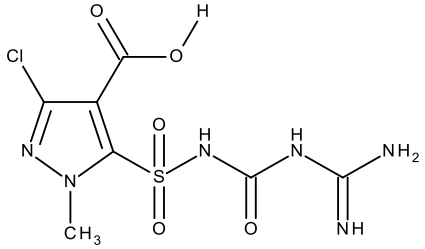
3-Chlorosulfonamide (CSA; Chlorosulfonamide acid; CSAA; MON5783)

IUPAC Name: 3-Chloro-1-methyl-5-sulfamoyl-pyrazole-4-carboxylic acid
CAS Name: Not reported
CAS Number: Not found
SMILES String: Cn1c(c(c(n1)Cl)C(=O)O)S(=O)(=O)N



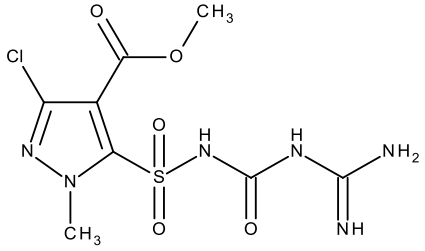
Halosulfuron acid guanidine (CSAG; Chlorosulfonamide acid guanidine; CSA-guanidine; CSA-g)

IUPAC Name: 5-(Carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylic acid
CAS Name: Not reported
CAS Number: Not found
SMILES String: Cn1c(c(c(n1)Cl)C(=O)O)S(=O)(=O)NC(=O)NC(=N)N

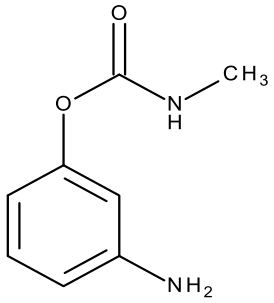


Halosulfuron ester guanidine (CSEG; Halosulfuron guanidine; Chlorosulfonamide guanidine; CSE-guanidine; CSE-g)

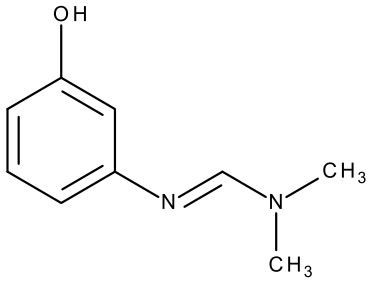
IUPAC Name: Methyl 5-(carbamimidoylcarbamoylsulfamoyl)-3-chloro-1-methyl-pyrazole-4-carboxylate
CAS Name: Not reported
CAS Number: Not found
SMILES String: Cn1c(c(c(n1)Cl)C(=O)OC)S(=O)(=O)NC(=O)NC(=N)N



M2 (CS_17365; AE F132316; Hoe 132316; 3-APMC)
IUPAC Name: 3-Aminophenyl methylcarbamate.
CAS Name: Not available.
CAS Number: Not available.
SMILES String: CNC(=O)Oc1cccc(c1)N



M3 (CS_17366; AE B035868; Hoe 133546; 3-HPDMF)
IUPAC Name: N'-(3-hydroxyphenyl)-N,N-dimethylimidoformamide.
CAS Name: Not available.
CAS Number: Not available.
SMILES String: CN(C)/C=N/c1cccc(c1)O



M4 (CS_17367; AE F132312; Hoe 132312; 3-HF)
IUPAC Name: N-(3-hydroxyphenyl)formamide.
CAS Name: Not available.
CAS Number: Not available.
SMILES String: c1cc(cc(c1)O)NC=O