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 water

Reports:	ECM: EPA MRID No.: 49798401. Shen, H. and T. Arndt. 2015. Development and Validation of a Method for the Determination of Halosulfuron-methyl (HSM) and its Degradates in Surface/Ground Water. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California, sponsored and submitted by Gowan Company, Yuma, Arizona; 203 pages. PTRL Study No: 2679W. Final report issued December 1, 2015. ILV: EPA MRID No.: 49983101. MacGregor, J.A. and E.S. Bodle. 2016. INDEPENDENT LABORATORY VALIDATION OF METHODS FOR THE DETERMINATION OF HALOSULFURON-METHYL (HSM) AND ITS DEGRADATES IN SURFACE/GROUND WATER 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; 201 pages. EAG Laboratories Project No: 334C-131. Final report issued July 27, 2016.
Document No.:	MRIDs 49798401 & 49983101
Guideline:	850.6100
Statements:	ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49798401). 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 49983101). 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. In the ILV, the composition of the ground water matrix was unclear. For analyte AP, method recoveries did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy for ground water matrix at the LOQ in the ILV and for surface water matrix at the LOQ and 10×LOQ in the ECM. For analytes CSA and CSAG, method RSDs in ground water matrix at the LOQ did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy for both ions in the ECM. In the ILV, linearity was not satisfactory for HS and CSA. In the ECM, the LOQ chromatograms for CSA and CSAG in both water matrices showed baseline interferences with peak resolution or integration. The LODs for the analytes were not reported in the ILV.
PC Code:	128721

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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: 2679W, 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 water using HPLC/MS/MS. In water, the method is quantitative for halosulfuron-methyl and RRE at the stated LOQ of 0.05 ppb and for CPSA, AP, HS, CSA, CSAG and CSEG at the LOQ of 0.2 ppb. The LOQs are greater than the lowest toxicological level of concern in water (EC25 = 0.045 μg ai/L; NOAEC = 0.023 μg ai/L). Characterized ground (well) water and natural surface water were used for the ECM validation; the specific water source type of the surface water was not reported. Characterized ground (well) water and natural surface water were used for the ILV validation; however, two sources of ground water were used for one ground water matrix. The ECM method was validated by the ILV with insignificant modifications to the sample processing procedure and the analytical method. The method for HSM/RRE/CPSA (CSE)/AP was validated by the ILV in the third trial with surface and ground water matrices. The method for CSA/HS was validated by the ILV in the second trial with surface and ground water matrices. The method for CSAG/CSEG was validated by the ILV in the first trial with surface and ground water matrices. All ILV data regarding repeatability, accuracy, and precision were satisfactory for all analytes in both matrices, except for AP in ground water. In the ILV, linearity was not satisfactory for HS and CSA. All ILV data regarding specificity were satisfactory for all analytes in both matrices; only quantitation ion chromatograms were provided. 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 AP in surface water and CSA and CSAG in ground water. All ECM data regarding specificity were satisfactory for all analytes in both matrices, except that baseline interferences affected the resolution and integration of the LOQ peaks for CSA and CSAG in both matrices.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	**MRID**		**EPA Review**	**Matrix**	**Method Date (dd/mm/yyyy)**	**Registrant**	**Analysis**	**Limit of Quantitation (LOQ)**
	Environmental Chemistry Method	**Independent Laboratory Validation**						
Halosulfuron-methyl (HSM)	49798401[2]	49983101[3]	Supplemental	Water	01/12/2015	Gowan Company, LLC Canyon Group LLC	LC/MS/MS	0.05 ppb
RRE								
CPSA (CSE)								0.2 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] In the ECM, ground (well) water (2706W-032; pH 7.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 960 ppm) and natural surface water (2440W-083; pH 6.3, total dissolved solids 68 ppm) were used(p. 22; Appendix C, pp. 184-185 of MRID 49798401). Sources not specified.

[3] In the ILV, two sources of ground (well) water were used for the ground water sample: PTRL West ground water (the matrix used in the ECM; 2706W-032; pH 7.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 960 ppm) and EAG Laboratories ground water (pH 7.95; hardness 136 mg/L as $CaCO_3$; p. 14; Appendices III-V, pp. 176-179 of MRID 49983101). The natural surface water (pH 7.00; hardness 64.0 mg/L as $CaCO_3$) was obtained from Tuckahoe Lake, Tuckahoe Lake State Park, Ridgely, Maryland.

I. Principle of the Method

Extraction procedure for HSM/RRE/CPSA (CSE)/AP: Water (100 mL; pH 5.5-7.5, adjusted with HCl or NaOH if necessary) in a 250-mL separatory funnel was fortified with 0.02 or 0.20 mL of 250 ng/mL HSM and RRE fortification solutions or 0.04 or 0.40 mL of 500 ng/mL CPSA (CSE) and AP fortification solutions (pp. 22-23, 30-31; Figure 1, p. 53 of MRID 49798401). Dichloromethane (40 mL) and sodium chloride (*ca*. 1 g) were mixed with the water sample via shaking vigorously for 2 minutes. After 5 minutes to allow the phases to separate, the dichloromethane layer (lower) was drained and filtered through a filter funnel containing *ca*. 5.5 g of sodium sulfate. The filter was rinsed with 10 mL of dichloromethane. The remaining water sample was extracted with ethyl acetate (40 mL) via shaking vigorously for 2 minutes. After 3 minutes to allow the phases to separate, the ethyl acetate layer was drained and combined with the dichloromethane extract. The combined extracts were reduced to *ca*. 5 mL via rotary evaporation at 150 mbar and 30°C. The residue was 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 a volume of *ca*. 0.2-0.4 mL at 30°C. The residue was reconstituted in 2.0 mL of acetonitrile:water (1:1, v:v) and mixed via vortex prior to LC/MS/MS analysis.

Extraction procedure for CSA/HS: Water (10 mL) in a 50-mL disposable plastic centrifuge tubes was fortified with 0.04 or 0.40 mL of 50 ng/mL HS and CSA fortification solutions (pp. 22-23, 30-32; Figure 2, p. 54 of MRID 49798401). The water was extracted with 1% acetic acid in acetonitrile (10 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 transferred to an autosampler vial for LC/MS/MS analysis.

Extraction procedure for CSAG/CSEG: Water (10 mL) in a 50-mL disposable plastic centrifuge tubes was fortified with 0.10 or 1.00 mL of 20 ng/mL CSAG and CSEG fortification solutions (pp. 22-23, 30, 32; Figure 3, p. 55 of MRID 49798401). The water was extracted with acetonitrile (10 mL), 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 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 of MRID 49798401). The following LC conditions were used (pp. 33-36): 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 varied depending on analyte.

HSM/RRE/CPSA (CSE)/AP: The following mobile phase gradient was used (pp. 33-34 of MRID 49798401): 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. 35-36 of MRID 49798401): 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 49798401): 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 and insignificant modifications to the analytical method for CSA/HS (pp. 19-23; Tables 1-3, pp. 31-33 of MRID 49983101). The LC/MS/MS instrument and parameters were the same as those of the ECM, with the exception that the mobile phase gradient for CSA/HS matched that of HSM/RRE/CPSA (CSE)/AP instead of that of CSAG/CSEG. 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 #8; 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 steps were 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; and in the method for CSA/HS, the fortification stock solution should be prepared in acetonitrile:water (1:1, v:v) to ensure stability and constant solubility of the CSA/HS analytes, especially the HS component (Appendix VII, p. 198 of MRID 49983101).

LOQ and LOD: In the ECM and ILV, the Limits of Quantification (LOQ) were 0.05 ppb for HSM and RRE and 0.2 ppb for CPSA (CSE), AP, HS, CSA, CSAG and CSEG (p. 10 of MRID 49798401. pp. 12, 20 of MRID 49983101). In the ECM, the Limits of Detection (LOD) were 0.01 ppb for HSM and RRE, 0.02 ppb for CPSA (CSE) and AP, 0.01 ppb for HS and CSA and 0.08 ppb for CSAG and CSEG. The LODs for the analytes were not reported in the ILV.

II. Recovery Findings

ECM (MRID 49798401): 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 0.05 ppb (LOQ) and 0.5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 0.2 ppb (LOQ) and 2.0 ppb (10×LOQ) in the surface water matrix, except for mean recoveries of AP which were 44-53% at the LOQ and 10×LOQ (ions combined; Tables I-II, pp. 45-50). Mean recoveries and RSDs were within guidelines for analysis of halosulfuron-methyl (HSM) and its transformation product RRE at fortification levels of 0.05 ppb (LOQ) and 0.5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 0.2 ppb (LOQ) and 2.0 ppb (10×LOQ) in the ground (well) water matrix, except for the RSDs of CSA and CSAG at the LOQ which were 25% (quantification ion) and 24% (confirmation ion), respectively. 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 HS, CSA (LOQ only) and CSAG (LOQ only) in the ground water matrix. Ground (well) water (2706W-032; pH 7.3, total dissolved solids 960 ppm) and natural surface water (2440W-083; pH 6.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 68 ppm) were characterized by Agvise Laboratories, Northwood, North Dakota (p. 22; Appendix C, pp. 184-185). The specific water source type of the surface water was not reported.

ILV (MRID 49983101): Mean recoveries and RSDs were within guidelines for analysis of halosulfuron-methyl (HSM) and its transformation product RRE at fortification levels of 0.05 ppb (LOQ) and 0.5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 0.2 ppb (LOQ) and 2.0 ppb (10×LOQ) in the surface water matrix (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 0.05 ppb (LOQ) and 0.5 ppb (10×LOQ), for its transformation products CPSA, AP, HS, CPA, CSAG and CSEG at 0.2 ppb (LOQ) and 2.0 ppb (10×LOQ) in the ground (well) water matrix, except for the mean recovery of AP at the LOQ which was 69.3-69.5% (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, except for RRE (LOQ only) in the surface water matrix. Two sources of ground (well) water were used for the ground water sample: PTRL West ground water (the matrix used in the ECM; 2706W-032; pH 7.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 960 ppm) and EAG Laboratories ground water (pH 7.95; hardness 136 mg/L as $CaCO_3$; see Reviewer's Comment #2; p. 14; Appendices III-V, pp. 176-179). The natural surface water (pH 7.00; hardness 64.0 mg/L as $CaCO_3$) was obtained from Tuckahoe Lake, Tuckahoe Lake State Park, Ridgely, Maryland. All water matrices were characterized by Agvise Laboratories, Northwood, North Dakota and EAG Laboratories. The method was validated with insignificant modifications to the sample processing procedure and the analytical method (pp. 13, 19-23; Tables 1-3, pp. 31-33). The method for HSM/RRE/CPSA (CSE)/AP was validated in the third trial with surface and ground water matrices (Appendix VII, pp. 196-199). The method for CSA/HS was validated in the second trial with surface and ground water matrices. The method for CSAG/CSEG was validated in the first trial with surface and ground water matrices.

Table 2. Initial Validation Method Recoveries for Halosulfuron-methyl (HSM) and Its Transformation Products RRE, CPSA, AP, HS, CSA, CPAG and CPEG in Water

Analyte[1]	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	84.4-98.0	90	6	6
	0.5	5	85.6-88.8	87	1	2
RRE	0.05 (LOQ)	5	92.8-102	97	3	4
	0.5	5	89.2-96.4	94	3	3
CPSA (CSE)	0.2 (LOQ)	5	86.5-114.0	104	10	10
	2.0	5	93.5-102.0	97	4	4
AP	0.2 (LOQ)	5	33.8-49.6	**44**	6	14
	2.0	5	38.3-62.5	**53**	9	17
HS	0.2 (LOQ)	5	74.0-94.0	87	8	9
	2.0	5	75.5-80.5	78	2	2
CSA	0.2 (LOQ)	5	68.0-95.5	85	13	15
	2.0	5	90.0-94.5	93	2	2
CSAG	0.2 (LOQ)	5	99.0-122	109	9	8
	2.0	5	81.5-91.5	87	4	5
CSEG	0.2 (LOQ)	5	112-125	118	5	4
	2.0	5	84.5-91.5	89	3	3
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	93.2-98.8	96	3	3
	0.5	5	84.0-87.2	86	1	2
RRE	0.05 (LOQ)	5	92.4-98.4	95	3	3
	0.5	5	90.8-94.4	93	2	2
CPSA (CSE)	0.2 (LOQ)	5	88.0-102	94	6	6
	2.0	5	92.5-98.5	97	3	3
AP	0.2 (LOQ)	5	31.8-53.0	**44**	8	18
	2.0	5	37.4-61.0	**51**	9	17
HS	0.2 (LOQ)	5	78.0-94.5	88	7	8
	2.0	5	76.0-78.5	77	1	1
CSA	0.2 (LOQ)	5	68.5-105	86	13	15
	2.0	5	87.0-93.5	91	3	3
CSAG	0.2 (LOQ)	5	95.0-132	115	13	11
	2.0	5	82.5-90.0	86	3	4
CSEG	0.2 (LOQ)	5	106-124	116	7	6
	2.0	5	86.0-96.0	91	4	5
	Ground (Well) Water[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	99.8-110	105	4	4
	0.5	5	98-107	102	3	3
RRE	0.05 (LOQ)	5	90.6-96.6	95	3	3
	0.5	5	90.0-93.2	92	1	2
CPSA (CSE)	0.2 (LOQ)	5	80.5-96.5	88	7	8
	2.0	5	83.5-87.5	85	2	2
AP	0.2 (LOQ)	5	58.5-69.0	63	4	7
	2.0	5	38.4-62.0	53	9	18

Analyte[1]	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
HS	0.2 (LOQ)	5	69.0-82.5	77	5	7
	2.0	5	79.0-96.0	84	7	8
CSA	0.2 (LOQ)	5	52.5-108	83	20	**25**
	2.0	5	87.5-93.0	91	2	3
CSAG	0.2 (LOQ)	5	87.0-116	96	12	12
	2.0	5	79.5-88.5	84	4	5
CSEG	0.2 (LOQ)	5	101-113	108	4	4
	2.0	5	89.0-97.5	92	4	4
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	93.2-103	98	4	4
	0.5	5	97.2-103	100	3	3
RRE	0.05 (LOQ)	5	91.6-99.0	96	3	3
	0.5	5	89.0-94.8	92	2	2
CPSA (CSE)	0.2 (LOQ)	5	79.0-95.0	88	7	8
	2.0	5	82.5-96.0	88	5	6
AP	0.2 (LOQ)	5	59.5-68.0	63	4	6
	2.0	5	38.6-62.5	53	9	18
HS	0.2 (LOQ)	5	62.5-88.0	70	10	15
	2.0	5	62.0-100	80	14	17
CSA	0.2 (LOQ)	5	94.0-116.0	103	9	9
	2.0	5	89.5-92.0	91	1	1
CSAG	0.2 (LOQ)	5	60.0-107	78	19	**24**
	2.0	5	81.5-89.5	86	3	3
CSEG	0.2 (LOQ)	5	110-119	112	5	4
	2.0	5	89.0-95.5	92	3	3

Data (uncorrected recovery results; pp. 37-38) were obtained from Tables I-II, pp. 45-50 of MRID 49798401.

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] Ground (well) water (2706W-032; pH 7.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 960 ppm) and natural surface water (2440W-083; pH 6.3, total dissolved solids 68 ppm) were characterized by Agvise Laboratories, Northwood, North Dakota (p. 22; Appendix C, pp. 184-185). The specific water source type of the surface water was 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.

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

Analyte[1]	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	82.6-92.8	88.0	3.66	4.16
	0.5	5	83.7-93.2	88.3	3.79	4.29
RRE	0.05 (LOQ)	5	114-124	120	4.04	3.37
	0.5	5	93.7-102	96.8	3.10	3.20
CPSA (CSE)	0.2 (LOQ)	5	79.8-106	92.4	10.4	11.3
	2.0	5	91.4-101	95.4	4.21	4.41
AP	0.2 (LOQ)	5	75.3-85.1	80.7	3.84	4.76
	2.0	5	73.2-81.9	78.0	3.20	4.10
HS	0.2 (LOQ)	5	96.2-102	99.2	2.10	2.12
	2.0	5	91.7-97.3	94.2	2.33	2.47
CSA	0.2 (LOQ)	5	87.4-101	93.8	5.61	5.98
	2.0	5	96.5-99.2	97.9	0.997	1.02
CSAG	0.2 (LOQ)	5	85.1-104	95.8	7.41	7.73
	2.0	5	87.1-92.5	89.9	2.20	2.45
CSEG	0.2 (LOQ)	5	109-126	117	7.89	6.74
	2.0	5	102-108	105	2.17	2.09
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	80.4-93.1	87.4	4.68	5.35
	0.5	5	84.9-90.4	87.0	2.24	2.57
RRE	0.05 (LOQ)	5	84.1-94.9	90.2	4.75	5.27
	0.5	5	96.2-104	98.6	3.13	3.17
CPSA (CSE)	0.2 (LOQ)	5	85.8-105	94.3	7.73	8.20
	2.0	5	90.8-97.2	93.5	2.62	2.80
AP	0.2 (LOQ)	5	70.4-85.4	79.0	5.72	7.24
	2.0	5	70.6-80.4	76.6	4.01	5.24
HS	0.2 (LOQ)	5	92.8-103	96.4	4.22	4.38
	2.0	5	93.9-96.4	95.4	0.992	1.04
CSA	0.2 (LOQ)	5	80.8-107	96.8	11.0	11.4
	2.0	5	94.4-102	98.6	3.26	3.31
CSAG	0.2 (LOQ)	5	98.0-107	102	3.81	3.74
	2.0	5	85.0-91.4	88.9	2.37	2.67
CSEG	0.2 (LOQ)	5	114-124	118	3.85	3.26
	2.0	5	108-110	109	0.837	0.766[4]
	Ground (Well) Water[2]					
	Quantitation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	77.7-87.3	85.2	4.18	4.91
	0.5	5	82.3-86.4	84.0	1.66	1.98
RRE	0.05 (LOQ)	5	89.1-96.7	92.9	2.95	3.18
	0.5	5	97.8-101	98.9	1.47	1.49
CPSA (CSE)	0.2 (LOQ)	5	93.1-106	100	5.86	5.86
	2.0	5	95.3-103	98.5	3.36	3.41
AP	0.2 (LOQ)	5	60.1-77.7	**69.3**	7.37	10.6
	2.0	5	70.0-89.8	75.3	8.19	10.9

Analyte[1]	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
HS	0.2 (LOQ)	5	95.8-102	98.9	2.35	2.38
	2.0	5	89.8-93.2	91.5	1.30	1.42
CSA	0.2 (LOQ)	5	76.8-91.0	85.1	5.73	6.73
	2.0	5	86.8-92.7	88.8	2.39	2.69
CSAG	0.2 (LOQ)	5	88.9-102	95.0	4.72	4.97
	2.0	5	87.0-89.6	88.4	1.03	1.17
CSEG	0.2 (LOQ)	5	98.6-114	107	5.64	5.27
	2.0	5	93.3-98.5	96.1	2.03	2.11
	Confirmation ion[3]					
Halosulfuron-methyl (HSM)	0.05 (LOQ)	5	80.3-89.1	86.1	3.74	4.34
	0.5	5	83.0-87.0	84.4	1.89	2.24
RRE	0.05 (LOQ)	5	89.0-97.0	92.8	2.90	3.13
	0.5	5	96.5-102	100	2.13	2.13
CPSA (CSE)	0.2 (LOQ)	5	88.0-100	93.9	5.65	6.02
	2.0	5	89.4-101	93.7	4.38	4.65
AP	0.2 (LOQ)	5	58.5-79.6	**69.5**	8.54	12.3
	2.0	5	66.9-88.3	74.2	8.19	11.0
HS	0.2 (LOQ)	5	97.6-110	103	4.93	4.79
	2.0	5	90.0-92.2	91.3	0.954	1.04
CSA	0.2 (LOQ)	5	85.8-105	93.9	9.69	10.3
	2.0	5	84.0-89.6	86.8	2.00	2.30
CSAG	0.2 (LOQ)	5	84.2-102	92.2	7.46	8.09
	2.0	5	85.9-91.0	88.2	1.88	2.13
CSEG	0.2 (LOQ)	5	103-115	108	4.76	4.41

Analyte[1]	**Fortification Level (ppb)**	**Number of Tests**	**Recovery Range (%)**	**Mean Recovery (%)**	**Standard Deviation (%)**	**Relative Standard Deviation (%)**
	2.0	5	92.8-98.1	96.2	2.02	2.10

Data (uncorrected recovery results; pp. 23-25) were obtained from Tables 4-35, pp. 34-65 of MRID 49983101.

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] Two sources of ground (well) water were used for the ground water sample: PTRL West ground water (the matrix used in the ECM; 2706W-032; pH 7.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 960 ppm) and EAG Laboratories ground water (pH 7.95; hardness 136 mg/L as $CaCO_3$; p. 14; Appendices III-V, pp. 176-179). The natural surface water (pH 7.00; hardness 64.0 mg/L as $CaCO_3$) was obtained from Tuckahoe Lake, Tuckahoe Lake State Park, Ridgely, Maryland. All water matrices were characterized by Agvise Laboratories, Northwood, North Dakota and EAG Laboratories.

[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 #8).

[4] The reviewer assumed that the value reported in the study report (7.68%) was a typographical error (see DER Attachment 2). The value listed is the RSD value calculated by the reviewer.

III. Method Characteristics

In the ECM and ILV, the LOQs were 0.05 ppb for HSM and RRE and 0.2 ppb for CPSA (CSE), AP, HS, CSA, CSAG and CSEG (pp. 10, 38-39, 43 of MRID 49798401; pp. 12, 20; Tables 4-35, pp. 34-65 of MRID 49983101). 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 0.01 ppb for HSM and RRE, 0.02 ppb for CPSA (CSE) and AP, 0.01 ppb for HS and CSA and 0.08 ppb for CSAG and CSEG. 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, except CSAG and CSEG. The LOD for CSAG and CSEG was 40% of the LOQ due to the sensitivity of the LC/MS/MS method. The LODs were 0.05 ng/mL for HSM and RRE and 0.01 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 Halosulfuron-methyl (HSM) and Its Transformation Products RRE, CPSA, AP, HS, CSA, CPAG and CPEG[1] in Water

			Halosulfuron-methyl HSM	RRE	CPSA (CSE)	AP	HS	CSA	CSAG	CSEG
Limit of Quantitation (LOQ)	ECM		0.05 ppb		0.2 ppb					
	ILV									
Limit of Detection (LOD)	ECM		0.01 ppb		0.02 ppb		0.01 ppb		0.08 ppb	
	ILV		Not reported							
Linearity (calibration curve r^2 and concentration range)	ECM[2]		$r^2 = 0.9981$ (Q) $r^2 = 0.9982$ (C)	$r^2 = 0.9987$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9975$ (Q) $r^2 = 0.9973$ (C)	$r^2 = 0.9987$ (Q) $r^2 = 0.9991$ (C)	$r^2 = 0.9999$ (Q) $r^2 = 0.9997$ (C)	$r^2 = 0.9982$ (Q) $r^2 = 0.9999$ (C)	$r^2 = 0.9992$ (Q) $r^2 = 0.9996$ (C)	$r^2 = 0.9988$ (Q) $r^2 = 0.9995$ (C)
		Range:	0.05-25 ng/mL		0.1-50 ng/mL		0.01-10 ng/mL		0.04-10 ng/mL	
	ILV[3]		$r^2 = 0.9999$ (Q)	$r^2 = 0.9987$ (Q)	$r^2 = 0.9996$ (Q)	$r^2 = 0.9971$ (Q)	$r^2 =$ **0.9886** (Q)	$r^2 =$ **0.9864** (Q)	$r^2 = 0.9998$ (Q)	$r^2 = 0.9992$ (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]	Surface Water:	Yes at LOQ and 10×LOQ.			**No**; mean recoveries **44**% at LOQ and **51-53**% at 10×LOQ.	Yes at LOQ and 10×LOQ.			
		Ground Water:	Yes at LOQ and 10×LOQ.					Yes at 10×LOQ. **No** at LOQ (Q), RSD = **25**%; Yes at LOQ (C).	Yes at 10×LOQ. **No** at LOQ (C), RSD = **24**%; Yes at LOQ (Q).	Yes at LOQ and 10×LOQ.
	ILV[5]	Surface Water:	Yes at LOQ and 10×LOQ.							
		Ground Water:	Yes at LOQ and 10×LOQ.			Yes at 10×LOQ. **No** at LOQ Mean = **69.3**% (Q), **69.5**% (C).	Yes at LOQ and 10×LOQ.			
Reproducible			Yes at LOQ and 10×LOQ in surface and ground water matrices.			Yes at LOQ and 10×LOQ in surface water matrix. Yes at	Yes at LOQ and 10×LOQ in surface and ground water matrices.			

					10×LOQ in ground water matrix; **No** at LOQ in ground water matrix.				
Specific	ECM	Surface Water:	Interferences were <10% of LOQ, based on peak height, at analyte retention times.	No matrix interferences were observed.		No matrix interferences were observed; however, analyte peak at LOD was barely resolved above the baseline.[6]	No matrix interferences were observed; however, analyte peak at LOQ not well-resolved from baseline.[7] Also, analyte peak at LOD was barely resolved above the baseline.[8]	No matrix interferences were observed; however, baseline noise interfered with analyte peak integration.[9] Also, peak at LOD was barely resolved above the baseline.[10]	No matrix interferences were observed.
		Ground Water:							
	ILV		Only quantitation ion chromatograms were provided.						
		Surface Water:	No matrix interferences were observed.	No matrix interferences were observed; however, baseline noise was significant near the analyte peak.[11]	No matrix interferences were observed.	No matrix interferences were observed.			
		Ground Water:			No matrix interferences were observed; however, minor baseline noise was observed near the analyte peak.				

Data were obtained from pp. 10, 12-15, 38-39, 43; Tables I-II, pp. 45-50 (recovery results); Figures 5-7, pp. 64-87 (reagent blanks and control water chromatograms); Figure 8, pp. 88-95 (calibration curves); Figures 12-15, pp. 120-151 (LOQ and 10×LOQ chromatograms); Figure 16, pp. 152-159 (LOD chromatograms) of MRID 49798401; 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 49983101. 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 49798401; 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 49983101; DER Attachment 2). The reviewer assumed that these curves were for the quantitation ion. The calibration curves were titled with “GW”, seeming to indicate that these were for the ground water set. Calibration standards were prepared in solvent (pp. 17-19).

4 In the ECM, ground (well) water (2706W-032; pH 7.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 960 ppm) and natural surface water (2440W-083; pH 6.3, total dissolved solids 68 ppm) were characterized by Agvise Laboratories, Northwood, North Dakota (p. 22; Appendix C, pp. 184-185 of MRID 49798401). The specific water source type of the surface water was not reported.

5 In the ILV, two sources of ground (well) water were used for the ground water sample: PTRL West ground water (the matrix used in the ECM; 2706W-032; pH 7.3, hardness 627 mg equiv. $CaCO_3$/L, total dissolved solids 960 ppm) and EAG Laboratories ground water (pH 7.95; hardness 136 mg/L as $CaCO_3$; p. 14; Appendices III-V, pp. 176-179 of MRID 49983101). The natural surface water (pH 7.00; hardness 64.0 mg/L as $CaCO_3$) was obtained from Tuckahoe Lake, Tuckahoe Lake State Park, Ridgely, Maryland. All water matrices were characterized by Agvise Laboratories, Northwood, North Dakota and EAG Laboratories.

6 Figure 16, p. 157 of MRID 49798401.

7 Figure 12, p. 124; Figure 14, p. 140 of MRID 49798401.

8 Figure 16, p. 156 of MRID 49798401.

9 Figure 12, p. 126; Figure 14, p. 142 of MRID 49798401.

10 Figure 16, p. 158 of MRID 49798401.

11 Figures 11-12, pp. 76-77 of MRID 49983101.

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 49798401 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 49798401 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 49798401.

2. In the ILV, two sources of ground (well) water were used for the ground water sample: PTRL West ground water and EAG Laboratories ground water, but only one set of ground water recovery data was reported (p. 14; Appendices III-V, pp. 176-179 of MRID 49983101). The ILV study authors did not specify the way in which these two ground water sources were used in the study, i.e. if the two samples were mixed evenly to create a combined ground water sample or if the samples were considered analogous and used independently in the study for various samples. The characteristics and constitution of the water matrix/matrices should be clear in the method validations.

3. In the ILV, the analysis of AP 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 in the ground water matrix (mean recoveries: 69.3% quantitation ion, 69.5% confirmation ion; Tables 10-11, pp. 40-41 of MRID 49983101). In a 2002 Aerobic Aquatic Metabolism study (MRID 45671701), 4,6-Dimethoxypyrimidin-2-amine (AP) was found to be a major degradate; the reviewed method does not meet the guideline requirements for analyzing this degradate.

   In the ECM, several compounds did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy at the stated LOQ and at higher concentrations in both water matrices. In the surface water matrix, the mean recoveries of AP were 44-53% at the LOQ and 10×LOQ (quantification and confirmation ions; Tables I-II, pp. 45-50 of MRID 49798401). In the ground water matrix, the RSDs of CSA and CSAG at the LOQ which were 25% (quantification ion) and 24% (confirmation ion), respectively. Regarding CSAG, 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.
   In a 2002 Aerobic Aquatic Metabolism study (MRID 45671701), 4,6-Dimethoxypyrimidin-2-amine (AP) and 3-Chloro-1-methyl-5-sulfamoyl-pyrazole-4-carboxylic acid (CSA) were found to be major degradates; does not meet the guideline requirements for analyzing these degradates.

4. In the ILV, linearity was not satisfactory for HS ($r^2 = 0.9886$) and CSA ($r^2 = 0.9864$; pp. 17-19; Figure 2, p. 67 of MRID 49983101). Linearity is satisfactory when $r^2 \geq 0.995$. Additionally, only one set of calibration cures was provided. The reviewer assumed that these curves were for the quantitation ion. The calibration curves were titled with "GW", seeming to indicate that these were for the ground water set, but 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.

5. 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 49983101). 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 49983101).

6. In the ECM, the LOQ chromatograms for CSA and CSAG in both water matrices showed baseline interferences with peak resolution or integration (Figure 12, pp. 124, 126; Figure 14, pp. 140, 142 of MRID 49798401).

7. 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, 38-39, 43 of MRID 49798401; pp. 12, 20; Tables 4-35, pp. 34-65 of MRID 49983101). 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, except CSAG and CSEG. The LOD for CSAG and CSEG was 40% of the LOQ due to the sensitivity of the LC/MS/MS method. The reviewer noted that the analyte peak at the LOD was barely resolved above the baseline for HS, CSA and CSAG (Figure 16, pp. 156-158). The LODs for the analytes were not reported in the ILV.

8. 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 49983101). 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.

9. In the ECM, matrix effects were evaluated in all matrices (p. 43; Table IV, p. 52 of MRID 49798401). The study authors determined that matrix effects (≥20%) were observed for CSEG (33.5%, surface water; 63.8% ground water), RRE (-21.2%, ground water), AP (-20.8%, ground water), HS (-22.1%, ground water) and CSAG (-31.9%, ground water). 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 prior to analysis.

10. The communications between the ILV and study developers and sponsors were detailed; communications involved failed trial discussions and suggested modifications (Appendix VII, pp. 199-200 of MRID 49983101).

11. 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 VII, pp. 198-199 of MRID 49983101). 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.

In the ECM, the total time required to perform the method (extraction and analysis) was *ca*. 30 hours (p. 39 of MRID 49798401). 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, and *ca*. 4 hours (processing) and *ca*. 4 hours (analysis and data processing) for the HS/CSA method or CSAG/CSEG method.

12. The ECM should be edited to account for the following critical steps 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; and in the method for CSA/HS, the fortification stock solution should be prepared in acetonitrile:water (1:1, v:v) to ensure stability and constant solubility of the CSA/HS analytes, especially the HS component (Appendix VII, p. 198 of MRID 49983101).

V. References

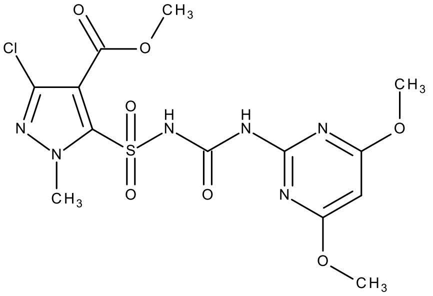
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

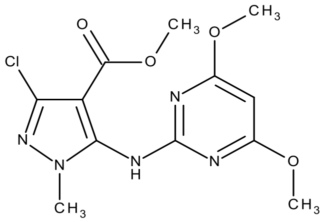
Halosulfuron-methyl (HSM; NC-319)

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



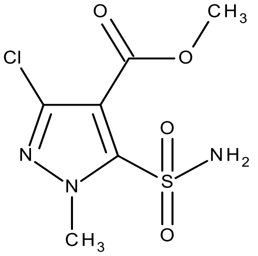
Halosulfuron-methyl rearrangement ester (RRE; HSMR)

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



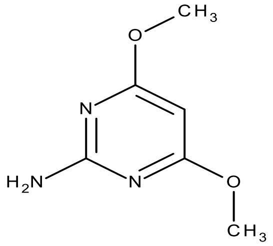
3-Chlorosulfonamide acid methyl ester (CPSA or CSE; Chlorosulfonamide)

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



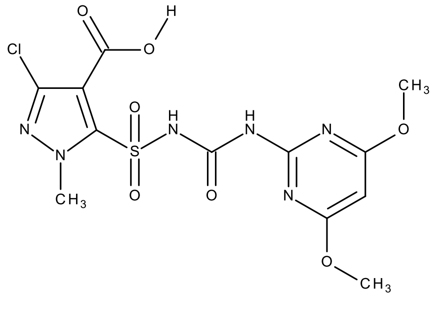
2-Amino-4,6-dimethoxypyrimidine (AP; ADMP; Aminopyrimidine; 620Pd-1)

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



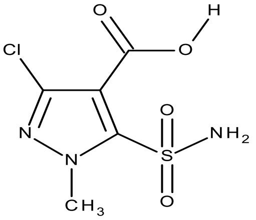
Halosulfuron acid (HS; Halosulfuron; 319-ACID; NC-319 ACID)

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



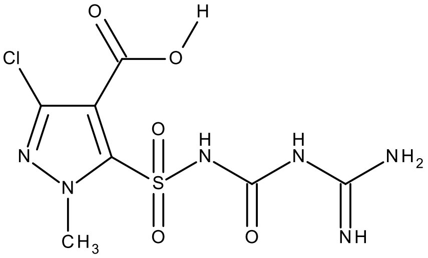
3-Chlorosulfonamide (CSA; Chlorosulfonamide acid; CSAA; MON5783)

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