

Analytical method for pymetrozine and its transformation products CGA 215525, CGA 300407, CGA 294849, CGA 371075, CGA 363431, CGA 359009, CGA 363430, CGA 255548, CGA 249257 and SYN 510306 in water

Reports: ECM: EPA MRID No.: 49673102. Lin, K. and S. Braid. 2015. Pymetrozine – Pymetrozine – Analytical Method GRM018.13A for the Determination of Pymetrozine and its Metabolites CGA 215525, CGA 300407, CGA 294849, CGA 371075, CGA 363431, CGA 359009, CGA 363430, CGA 255548, CGA 249257 and SYN 510306 in Water – Analytical Method. Syngenta Report No.: GRM018.13A and Task No.: TK0250015. Report prepared, sponsored, and submitted by Syngenta Crop Protection LLC, Greensboro, North Carolina; 210 pages. Final report issued June 16, 2015.

ILV: EPA MRID No. 49673101. Guo, D. 2015. Pymetrozine – Pymetrozine – Independent Laboratory Validation of Analytical Method (GRM018.13A) for the Determination of Pymetrozine and its Metabolites CGA 215525, CGA 300407, CGA 294849, CGA 371075, CGA 363431, CGA 359009, CGA 363430, CGA 255548, CGA 249257 and SYN 510306 in Water – Final ILV Report. PASC Report No.: PASC-REP-0579 and Project No.: 141-1114. Task No.: TK0250016. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey, sponsored and submitted by Syngenta Crop Protection LLC, Greensboro, North Carolina; 467 pages. Final report issued June 9, 2015.

Document No.: MRIDs 49673102 & 49673101

Guideline: 850.6100

Statements: ECM: The study was conducted with no claim of compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49673102). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). A statement of Quality Assurance and Authenticity was not included.

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49673101). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was not included.

Classification: This analytical method is classified as Supplemental. A few of the ECM correlation coefficients and almost all of the ILV correlation coefficients were unsatisfactory ($r^2 < 0.995$). Method modifications were performed by the ILV in order to complete a successful analysis of CGA 249257.

PC Code: 101103

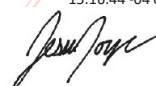
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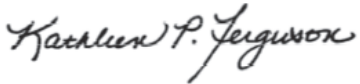
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Executive Summary

This analytical method, Syngenta Crop Protection Analytical Method GRM018.13A, is designed for the quantitative determination of pymetrozine (CGA 215944) and its transformation products CGA 215525, CGA 300407, CGA 294849, CGA 371075, CGA 363431, CGA 359009, CGA 363430, CGA 255548, CGA 249257 and SYN 510306 in water using LC/MS/MS at the LOQ of 0.05 µg/L. The LOQ is less than the lowest toxicological level of concern in water for all analytes. The ILV validated the method for all analytes in the first trial with modifications to sample preparation, injection volume, mobile phase gradient (for CGA 249257 in groundwater only) and mass spectrometer parameters; multiple ILV modifications to the ECM method were necessary to generate a successful trial for CGA 249257. The ILV modifications were not included in an amended final ECM report. The ground and surface water matrices of the ECM were the same as those of the ILV. A few of the ECM correlation coefficients and almost all of the ILV correlation coefficients were unsatisfactory ($r^2 < 0.995$). The specificity of the method was considered satisfactory based on matrix interferences; however, the reviewer noted significant baseline noise in most of the LOQ chromatograms of the ECM and ILV.

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						
Pymetrozine	49673102 ²	49673101 ³	Supplemental	Water	16/06/2015	Syngenta Crop Protection LLC	LC/MS/ MS	0.05 µg/L
CGA 215525								
CGA 300407								
CGA 294849								
CGA 371075								
CGA 363431								
CGA 359009								
CGA 363430								
CGA 255548								
CGA 249257								
SYN 510306								

1 Pymetrozine = (E)-4,5-Dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one; CGA 215525 = 4-Amino-6-methyl-2,5-dihydro-1,2,4-triazin-3-one; CGA 300407 = 3-Pyridinecarbaldehyde; CGA 294849 = 4-Amino-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione; CGA 371075 = 4,6-Dimethyl-2H-1,2,4-triazine-3,5-dione; CGA 363431 = 5-Hydroxy-6-methyl-4-[[1-(6-oxo-1,6-dihydro-pyridin-3-yl)-meth-(E)-ylidene]-amino]-4,5-dihydro-2H-[1,2,4]triazin-3-one; CGA 359009 = 4,5-Dihydro-5-hydroxy-6-methyl-4-[(3-pyridinylmethylene)amino]-1,2,4-triazine-3-(2H)-one; CGA 363430 = 6-Methyl-4-[[1-(6-oxo-1,6-dihydro-pyridin-3-yl)-meth-(E)-ylidene]-amino]-2H-[1,2,4]triazine-3,5-dione; CGA 255548 = 6-Oxo-1,6-dihydro-pyridine-3-carbaldehyde; CGA 249257 = 6-Methyl-4,5-dihydro-2H-1,2,4-triazin-3-one; SYN 510306 = 6-Methyl-4-[(6-oxo-1,6-dihydro-pyridin-3-ylmethylene)-amino]-4,5-dihydro-2H-[1,2,4]triazin-3-one.

2 In the ECM, the surface (local lake) water (pH 7.3, total hardness 27 CaCO₃ mg/L) and ground (local well) water (pH 7.5, total hardness 59 mg CaCO₃ mg/L) matrices were partially characterized (Table 1, p. 36 of MRID 49673102). The soil sources were not further specified.

3 In the ILV, the surface water (Agvise Lab # 12-156; PASC Sample ID 150104-1; Syngenta Study # TK0048240; Sample ID RIMV00312-001 Surface; pH 7.3, total hardness 27 mg CaCO₃ mg/L, total dissolved solids 58 ppm) and groundwater (Agvise Lab # 12-157; PASC Sample ID 150104-2; Syngenta Study # TK0048240; Sample ID RIMV00312-002 Ground; pH 7.5, total hardness 59 CaCO₃ mg/L, total dissolved solids 122 ppm) matrices were characterized (p. 25; Table 1, p. 51; Appendix 3, pp. 442-453 of MRID 49673101). These two matrices were the same as those used in the ECM; ILV soil samples were provided by the sponsor.

I. Principle of the Method

Water (40 mL) was filtered, fortified, as necessary, with the freshly-prepared combined fortification solution and mixed via vortex for 30 seconds (pp. 15-16 of MRID 49673102). Any further clean-up, based on the analyte, is described below. The Method Flow Chart was provided in the study report (Appendix 3, p. 207).

For analysis of CGA 294849 and CGA 371075, an aliquot (1.0 mL) was transferred to an autosampler vial and analyzed by LC/MS/MS (p. 16 of MRID 49673102). A filter (0.22 µm PVDF filter) was used if visible particles were observed.

For analysis of CGA 249257, an aliquot (1.0 mL) was transferred to an autosampler vial and analyzed by LC/MS/MS (pp. 16-17 of MRID 49673102). If there were “sensitivity issues”, an aliquot (10.0 mL) of the 40-mL, fortified water sample was mixed with 10 mL of methanol. The mixture was reduced to a total volume of 1 mL of less under a stream of nitrogen or air at 40-50°C. Adjust the volume to 1.0 mL with ultrapure water, if necessary. After centrifugation (8000 rpm for *ca.* 3 minutes) at 10°C, the supernatant was transferred to an injection vial for LC/MS/MS analysis. The method detailed the following optional analytical method for CGA 249257. An aliquot (10.0 mL) of the 40-mL, fortified water sample was transferred to a 50-mL centrifuge tube and mixed with 10 mL of acetonitrile via vortex. The mixture was extracted with Quechers packet buffered AOAC method (Agilent Part No. 5982-5755) or approximately 6 g magnesium sulfate and 1.5 g sodium acetate. The mixture was vortexed and shaken by hand occasionally (venting must be done to avoid pressure build-up). After shaking on a mechanical shaker for *ca.* 5 minutes, the mixture was centrifuged (5000 rpm for 5 minutes at 10°C). The top layer was decanted into a 100-mL round bottom flask. The remaining solution was extracted twice with 10 mL of acetonitrile in the same manner as just described. The study method noted that the aqueous layer has a tendency to become more absorbed with the magnesium sulfate in each extraction, and the acetonitrile layer is being absorbed into the aqueous layer. The combined acetonitrile extracts were evaporated to dryness using a rotovap or nitrogen stream at 40°C. The residue was reconstituted in either 1 mL (low recovery samples) or 10 mL (high recovery samples) of methanol:ultrapure water (10:90, v:v). After hand-swirling and brief sonication, an aliquot (1.0 mL) was transferred to an autosampler vial and analyzed by LC/MS/MS.

For analysis of pymetrozine (CGA 215944), CGA 215525, CGA 300407, CGA 363431, CGA 359009, CGA 363430, CGA 255548 and SYN 510306, an aliquot (20 mL) of the 40-mL, fortified water sample was mixed with 10 µL of ammonium hydroxide (29% w/v) via vortex for 30 seconds (p. 17 of MRID 49673102). An aliquot (*ca.* 1.0 mL) was immediately transferred to an autosampler vial and analyzed by LC/MS/MS.

The method contained the following experimental precautions: 1) bottles Optima Grade ultrapure water and methanol were used for the LC mobile phase; 2) high fortification samples should be diluted prior to LC analysis to prevent contamination and carry-over; 3) LC analysis should be completed immediately due to instability of analytes in aqueous solutions; and 4) instrument warm-up should be completed with at least 5 warm-up samples (p. 18 of MRID 49673102).

All samples were analyzed using a Waters Aquity UPLC I class LC and AB Sciex 5500 Qtrap MS (pp. 18-27 of MRID 49673102).

The following conditions were used for analysis of CGA 294849 and CGA 371075: ACE 5 C18 PFP column (3.0 mm x 100 mm, 5 μ m; column temperature ambient); a mobile phase of (A) ultrapure water and (B) methanol [percent A:B at 0-1 min. 100:0, 5.5 min. 50:50, 7.0-8.5 min. 5:95, 8.6-12 min. 100:0]; injection volume 50-75 μ L; MS/MS detection using TurboIonSpray (600°C); negative ion mode; and multiple reaction monitoring (MRM). Expected retention times were 3.6 and 5.3 minutes for CGA 294849 and CGA 371075, respectively. Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored: m/z 141.0 \rightarrow 42.0 (Q) and m/z 141.0 \rightarrow 124.9 (C) for CGA 294849 and m/z 140.0 \rightarrow 42.0 (Q) and m/z 140.0 \rightarrow 68.0 (C) for CGA 371075.

The following conditions were used for analysis of pymetrozine (CGA 215944), CGA 215525, CGA 300407, CGA 363431, CGA 359009, CGA 363430, CGA 255548 and SYN 510306: Agilent Zorbax SB-Aq column (4.5 mm x 50 mm, 3.5 μ m; column temperature ambient); a mobile phase of (A) 0.003-0.006% ammonium hydroxide in ultrapure water and (B) 0.003-0.006% ammonium hydroxide in methanol [percent A:B at 0-1 min. 100:0, 5.5 min. 50:50, 7.0-15.0 min. 5:95, 15.1-25 min. 100:0]; injection volume 50-75 μ L; MS/MS detection using TurboIonSpray (620°C); positive ion mode; and multiple reaction monitoring (MRM). Expected retention times were 7.4, 4.1, 6.4, 5.5, 6.9, 5.4, 4.6 and 6.3 minutes for pymetrozine (CGA 215944), CGA 215525, CGA 300407, CGA 363431, CGA 359009, CGA 363430, CGA 255548 and SYN 510306, respectively. Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored: m/z 217.8 \rightarrow 105.0 (Q) and m/z 217.8 \rightarrow 78.0 (C) for pymetrozine (CGA 215944); m/z 129.0 \rightarrow 44.9 (Q) and m/z 129.0 \rightarrow 129.0 (88.1) (C) for CGA 215525; m/z 108.0 \rightarrow 80.2 (Q) and m/z 108.0 \rightarrow 53.0 (C) for CGA 300407; m/z 250.1 \rightarrow 121.1 (Q) and m/z 250.1 \rightarrow 103.1 (C) for CGA 363431; m/z 234.0 \rightarrow 105.2 (Q) and m/z 234.0 \rightarrow 92.1 (C) for CGA 359009; m/z 248.0 \rightarrow 121.1 (Q) and m/z 248.0 \rightarrow 103.1 (C) for CGA 363430; m/z 124.0 \rightarrow 106.1 (Q) and m/z 124.0 \rightarrow 78.1 (C) for CGA 255548; and m/z 234.0 \rightarrow 121.3 (Q) and m/z 234.0 \rightarrow 103.1 (C) for SYN 510306.

The following conditions were used for analysis of CGA 249257: ACE Excel 3 C18 AR column (3.0 mm x 150 mm, 5 μ m; column temperature 40°C); a mobile phase of (A) 0.05% acetic acid in ultrapure water and (B) methanol [percent A:B at 0-1 min. 95:5, 5.5 min. 50:50, 7.0-10 min. 5:95, 10.1-14 min. 95:5]; injection volume 50-75 μ L; MS/MS detection using TurboIonSpray (550°C); positive ion mode; and multiple reaction monitoring (MRM). Expected retention time was 3.6 minutes for CGA 249257. Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored: m/z 113.8 \rightarrow 72.9 (Q) and m/z 113.8 \rightarrow 70.8 (C) for CGA 249257. Optional alternative LC conditions for CGA 249257 were included: Agilent Pursuit XRs 3 Diphenyl column (4.6 mm x 100 mm; column temperature 40°C); a mobile phase of (A) 5 mM ammonium acetate in ultrapure water and (B) methanol [percent A:B at 0-1 min. 95:5, 5.5 min. 50:50, 7.0-10 min. 5:95, 10.1-14 min. 95:5]; injection volume 50-75 μ L. Expected retention time was 6.4 minutes for CGA 249257.

In the ILV, the ECM method was performed with the following modifications: 1) samples were centrifuged (*ca.* 8000 rpm for *ca.* 3 minutes at 10°C) prior to transferring to an autosampler vial for LC/MS/MS analysis; no filtration was used; 2) for the clean-up procedure for all analytes, but CGA 294849, CGA 371075 and CGA 249257, 0.1 mL of 10% ammonium hydroxide, instead of 0.01 mL of ammonium hydroxide (29% w/v), was used to lower the pH of the sample; 3) for CGA 249257 in groundwater samples, the extraction procedure was followed which was specified for “sensitivity issues” in the ECM (reconstituted to 1 mL for low recovery samples and 10 mL for high recovery samples; 4) for CGA 249257 in surface water samples, the same extraction procedure was followed as for the groundwater samples, except that the sample was evaporated to 0.5 mL or less and reconstituted with 0.05% acetic acid in water (1 mL for low recovery samples and 2 mL for high recovery samples); 5) for CGA 249257 in groundwater samples, the LC mobile phase gradient was modified to the following due to the reconstitution solution change to 10% methanol in water: percent A:B at 0-1 min. 88:12, 5.5 min. 50:50, 7.0-10.0 min. 5:95, 10.1-14 min. 88:22; and 6) the calibration standard concentrations were changed, but the range remained the same (pp. 31-33; Appendix 5, pp. 465-466 of MRID 49673101). The ILV report also noted that a 75- μ L injection volume was used for all analytes. All samples were analyzed using a Waters Aquity UPLC LC and Applied Biosystems Sciex API 6500 triple quadrupole MS (pp. 33-39). Expected retention times were 3.4 and 5.2 minutes for CGA 294849 and CGA 371075, respectively. Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored: m/z 141.0 \rightarrow 42.0 (Q) and m/z 141.0 \rightarrow 124.9 (C) for CGA 294849 and m/z 140.1 \rightarrow 42.1 (Q) and m/z 140.1 \rightarrow 67.8 (C) for CGA 371075. Expected retention times were 7.4, 4.1, 6.5, 5.8, 7.0, 5.5, 4.6 and 6.3 minutes for pymetrozine (CGA 215944), CGA 215525, CGA 300407, CGA 363431, CGA 359009, CGA 363430, CGA 255548 and SYN 510306, respectively. Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored: m/z 217.8 \rightarrow 105.0 (Q) and m/z 217.8 \rightarrow 78.0 (C) for pymetrozine (CGA 215944); m/z 129.0 \rightarrow 45.0 (Q) and m/z 129.0 \rightarrow 88.1 (C) for CGA 215525; m/z 108.2 \rightarrow 80.2 (Q) and m/z 108.2 \rightarrow 53.2 (C) for CGA 300407; m/z 250.1 \rightarrow 121.1 (Q) and m/z 250.1 \rightarrow 103.1 (C) for CGA 363431; m/z 234.0 \rightarrow 105.2 (Q) and m/z 234.0 \rightarrow 92.1 (C) for CGA 359009; m/z 248.0 \rightarrow 121.1 (Q) and m/z 248.0 \rightarrow 103.1 (C) for CGA 363430; m/z 124.0 \rightarrow 106.1 (Q) and m/z 124.0 \rightarrow 78.1 (C) for CGA 255548; and m/z 234.0 \rightarrow 121.3 (Q) and m/z 234.0 \rightarrow 103.1 (C) for SYN 510306. The primary LC conditions were used for CGA 249257, except for the modification mentioned above for groundwater. Expected retention times for CGA 249257 were 3.0 and 3.7 minutes for ground and surface water samples, respectively. Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored: m/z 114.0 \rightarrow 73.1 (Q) and m/z 114.1 \rightarrow 71.1 (C). Some additional minor modifications of the LC/MS/MS conditions were reported. Ultrapure water was not specified.

The Limit of Quantitation (LOQ) and Limit of Detection (LOD) for all analytes were the same in the ECM and ILV at 0.05 μ g/L (0.05 ppb) and 0.025 ng/mL, respectively (pp. 12, 31 of MRID 49673102; pp. 17, 40, 48; Figures 12-55, pp. 88-109 of MRID 49673101).

II. Recovery Findings

ECM (MRID 49673102): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of pymetrozine, CGA 294849, CGA 215525, CGA 300407, CGA 371075, CGA 363431, CGA 359009, CGA 363430, CGA 255548, CGA 249257 and SYN 510306 in groundwater and surface water at fortification levels of 0.05 $\mu\text{g/L}$ (LOQ) and 0.5 $\mu\text{g/L}$ [10x LOQ; quantitation and confirmatory ions; Table 2, pp. 37-47; DER Attachment 2 (calculation of mean, standard deviation and relative standard deviation values (RSD))]. Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. The ground (local well) water (pH 7.5, total hardness 59 mg CaCO_3 mg/L) and surface (local lake) water (pH 7.3, total hardness 27 CaCO_3 mg/L) matrices were partially characterized (Table 1, p. 36). The water sources were not further specified.

ILV (MRID 49673101): Mean recoveries and RSDs were within guideline requirements for analysis of pymetrozine, CGA 294849, CGA 215525, CGA 300407, CGA 371075, CGA 363431, CGA 359009, CGA 363430, CGA 255548, CGA 249257 and SYN 510306 in groundwater and surface water at fortification levels of 0.05 $\mu\text{g/L}$ (LOQ) and 0.5 $\mu\text{g/L}$ (10x LOQ; pp. 41-46; Tables 3-24, pp. 53-74). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. Characterized surface water (Agvise Lab # 12-156; PASC Sample ID 150104-1; Syngenta Study # TK0048240; Sample ID RIMV00312-001 Surface; pH 7.3, total hardness 27 mg CaCO_3 mg/L, total dissolved solids 58 ppm) and groundwater (Agvise Lab # 12-157; PASC Sample ID 150104-2; Syngenta Study # TK0048240; Sample ID RIMV00312-002 Ground; pH 7.5, total hardness 59 CaCO_3 mg/L, total dissolved solids 122 ppm) matrices were used in the study (p. 25; Table 1, p. 51; Appendix 3, pp. 442-453). These two matrices were the same as those used in the ECM. Two other matrices were reported, as well: surface water (Agvise Lab # 15-33; PASC Sample ID 150208-1; Syngenta Study # N/A; Sample ID RIMV00115-0001; pH 8.6, total hardness 21 mg CaCO_3 mg/L, total dissolved solids 84 ppm) and groundwater (Agvise Lab # 15-34; PASC Sample ID 150208-2; Syngenta Study # N/A; Sample ID RIMV00115-0002; pH 7.7, total hardness 54 CaCO_3 mg/L, total dissolved solids 156 ppm). These two other matrices did not appear to be the matrix samples used in the method validation, based on their Sample ID. All characterization was performed by Agvise Laboratories, Northwood, North Dakota; soil samples were provided by the sponsor. The method was validated for all analytes in the first trial with modifications to sample preparation, injection volume, mobile phase gradient (for CGA 249257 in groundwater only) and mass spectrometer parameters (pp. 31-39). These ILV modifications were not included in an amended final ECM report.

Table 2. Initial Validation Method Recoveries for Pymetrozine and Its Transformation Products in Water (ECM) ^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Quantitation ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	86-95	91	4	5
	0.5	5	95-103	100	3	3
CGA 215525	0.05 (LOQ)	5	88-99	93	5	6
	0.5	5	100-107	104	3	2
CGA 300407	0.05 (LOQ)	5	94-100	98	2	2
	0.5	5	76-89	80	5	6
CGA 363431	0.05 (LOQ)	5	80-92	86	5	5
	0.5	5	86-93	90	3	3
CGA 359009	0.05 (LOQ)	5	83-96	89	5	5
	0.5	5	90-95	93	2	2
CGA 363430	0.05 (LOQ)	5	98-105	102	3	3
	0.5	5	72-80	77	3	4
CGA 255548	0.05 (LOQ)	5	84-106	96	10	10
	0.5	5	100-105	102	2	2
SYN 510306	0.05 (LOQ)	5	86-96	91	4	5
	0.5	5	97-107	100	4	4
CGA 294849	0.05 (LOQ)	5	90-102	97	5	6
	0.5	5	96-103	100	3	3
CGA 371075	0.05 (LOQ)	5	88-100	95	5	5
	0.5	5	88-99	94	4	4
CGA 249257	0.05 (LOQ)	5	82-98	92	6	7
	0.5	5	91-94	93	1	1
Confirmatory ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	88-93	91	2	2
	0.5	5	92-100	97	3	3
CGA 215525	0.05 (LOQ)	5	82-95	86	6	6
	0.5	5	89-100	94	4	4
CGA 300407	0.05 (LOQ)	5	93-100	97	3	3
	0.5	5	88-94	91	2	3
CGA 363431	0.05 (LOQ)	5	89-96	92	3	3
	0.5	5	82-92	89	4	4
CGA 359009	0.05 (LOQ)	5	84-94	90	5	5
	0.5	5	91-101	97	4	4
CGA 363430	0.05 (LOQ)	5	91-106	98	6	6
	0.5	5	75-95	88	8	9
CGA 255548	0.05 (LOQ)	5	92-102	96	4	4
	0.5	5	93-102	99	4	4
SYN 510306	0.05 (LOQ)	5	89-100	95	4	4
	0.5	5	86-105	98	7	7
CGA 294849	0.05 (LOQ)	5	91-98	94	3	3
	0.5	5	96-102	99	3	3
CGA 371075	0.05 (LOQ)	5	81-89	86	3	4

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5	5	97-103	100	2	2
CGA 249257	0.05 (LOQ)	5	89-105	96	6	7
	0.5	5	91-94	92	1	1
Groundwater						
Quantitation ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	91-96	94	2	2
	0.5	5	92-97	95	2	2
CGA 215525	0.05 (LOQ)	5	82-98	92	6	7
	0.5	5	93-106	101	5	5
CGA 300407	0.05 (LOQ)	5	94-99	97	2	2
	0.5	5	90-109	98	8	8
CGA 363431	0.05 (LOQ)	5	72-86	79	6	7
	0.5	5	85-97	90	5	6
CGA 359009	0.05 (LOQ)	5	90-102	97	4	5
	0.5	5	90-94	92	2	2
CGA 363430	0.05 (LOQ)	5	103-110	106	3	2
	0.5	5	103-110	106	3	2
CGA 255548	0.05 (LOQ)	5	89-94	91	3	3
	0.5	5	99-101	100	1	1
SYN 510306	0.05 (LOQ)	5	91-98	94	3	3
	0.5	5	87-105	98	7	7
CGA 294849	0.05 (LOQ)	5	97-107	103	4	4
	0.5	5	93-102	98	3	3
CGA 371075	0.05 (LOQ)	5	88-100	94	5	5
	0.5	5	98-102	100	2	2
CGA 249257	0.05 (LOQ)	5	82-123	92	17	19
	0.5	5	73-98	86	12	14
Confirmatory ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	87-94	91	3	3
	0.5	5	91-95	93	2	2
CGA 215525	0.05 (LOQ)	5	76-94	87	8	9
	0.5	5	92-105	100	5	5
CGA 300407	0.05 (LOQ)	5	88-103	95	6	6
	0.5	5	96-109	103	5	5
CGA 363431	0.05 (LOQ)	5	70-80	86	4	5
	0.5	5	82-95	89	5	6
CGA 359009	0.05 (LOQ)	5	94-103	99	3	3
	0.5	5	88-97	92	4	4
CGA 363430	0.05 (LOQ)	5	82-98	89	6	7
	0.5	5	75-95	86	8	9
CGA 255548	0.05 (LOQ)	5	95-99	98	2	2
	0.5	5	88-102	95	5	5
SYN 510306	0.05 (LOQ)	5	90-100	96	4	4
	0.5	5	86-98	93	5	6
CGA 294849	0.05 (LOQ)	5	100-106	102	2	2
	0.5	5	97-102	99	2	2

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
CGA 371075	0.05 (LOQ)	5	87-97	94	4	4
	0.5	5	95-101	98	2	2
CGA 249257	0.05 (LOQ)	5	77-104	88	10	11
	0.5	5	83-111	95	10	11

Data (uncorrected recovery results, pp. 28-30) were obtained from Table 2, pp. 37-47 of MRID 49673102 and DER Attachment 2 (calculation of mean, s.d. and RSD values).

* Not calculated, n = 2.

- The surface (local lake) water (pH 7.3, total hardness 27 CaCO₃ mg/L) and ground (local well) water (pH 7.5, total hardness 59 mg CaCO₃ mg/L) matrices were partially characterized (Table 1, p. 36).
- Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored for each analyte: *m/z* 141.0→42.0 (Q) and *m/z* 141.0→124.9 (C) for CGA 294849; *m/z* 140.0→42.0 (Q) and *m/z* 140.0→68.0 (C) for CGA 371075; *m/z* 217.8→105.0 (Q) and *m/z* 217.8→78.0 (C) for pymetrozine (CGA 215944); *m/z* 129.0→44.9 (Q) and *m/z* 129.0→129.0 (88.1) (C) for CGA 215525; *m/z* 108.0→80.2 (Q) and *m/z* 108.0→53.0 (C) for CGA 300407; *m/z* 250.1→121.1 (Q) and *m/z* 250.1→103.1 (C) for CGA 363431; *m/z* 234.0→105.2 (Q) and *m/z* 234.0→92.1 (C) for CGA 359009; *m/z* 248.0→121.1 (Q) and *m/z* 248.0→103.1 (C) for CGA 363430; *m/z* 124.0→106.1 (Q) and *m/z* 124.0→78.1 (C) for CGA 255548; *m/z* 234.0→121.3 (Q) and *m/z* 234.0→103.1 (C) for SYN 510306; and 113.8→72.9 (Q) and *m/z* 113.8→70.8 (C) for CGA 249257 (pp. 18-27).

Table 3. Independent Validation Method Recoveries for Pymetrozine and Its Transformation Products in Water^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Quantitation ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	87-102	95	7	7
	0.5	5	99-113	105	6	6
CGA 215525	0.05 (LOQ)	5	93-114	105	8	7
	0.5	5	102-115	108	7	6
CGA 300407	0.05 (LOQ)	5	87-109	99	10	10
	0.5	5	108-120	110	6	6
CGA 363431	0.05 (LOQ)	5	76-95	89	8	9
	0.5	5	104-112	108	4	3
CGA 359009	0.05 (LOQ)	5	78-102	94	10	10
	0.5	5	101-117	107	7	6
CGA 363430	0.05 (LOQ)	5	79-96	90	8	9
	0.5	5	99-112	105	6	5
CGA 255548	0.05 (LOQ)	5	84-111	99	12	12
	0.5	5	103-117	109	6	6
SYN 510306	0.05 (LOQ)	5	84-101	94	7	7
	0.5	5	100-116	106	7	6
CGA 294849	0.05 (LOQ)	5	80-100	89	9	10
	0.5	5	96-106	100	4	4
CGA 371075	0.05 (LOQ)	5	102-116	109	5	5
	0.5	5	90-100	96	5	5
CGA 249257	0.05 (LOQ)	5	82-98	92	6	7
	0.5	5	91-94	93	1	1
Confirmatory ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	86-104	96	7	7
	0.5	5	98-113	105	6	6
CGA 215525	0.05 (LOQ)	5	96-121	111	10	9
	0.5	5	102-116	109	5	5
CGA 300407	0.05 (LOQ)	5	90-125	105	14	13
	0.5	5	100-118	109	7	6
CGA 363431	0.05 (LOQ)	5	59-88	80	12	15
	0.5	5	103-111	107	3	3
CGA 359009	0.05 (LOQ)	5	81-106	95	9	10
	0.5	5	99-116	106	7	6
CGA 363430	0.05 (LOQ)	5	85-101	94	7	7
	0.5	5	98-110	104	5	5
CGA 255548	0.05 (LOQ)	5	70-114	91	18	20
	0.5	5	99-112	104	6	5
SYN 510306	0.05 (LOQ)	5	91-109	101	7	7
	0.5	5	100-115	106	7	6
CGA 294849	0.05 (LOQ)	5	83-100	93	7	8
	0.5	5	93-102	98	4	4
CGA 371075	0.05 (LOQ)	5	88-107	96	7	8

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5	5	91-102	95	4	5
CGA 249257	0.05 (LOQ)	5	89-105	96	7	7
	0.5	5	91-94	92	1	1
Groundwater						
Quantitation ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	100-106	103	2	2
	0.5	5	102-127	111	9	9
CGA 215525	0.05 (LOQ)	5	100-120	110	8	7
	0.5	5	93-124	102	12	12
CGA 300407	0.05 (LOQ)	5	81-102	93	9	10
	0.5	5	99-132	111	13	12
CGA 363431	0.05 (LOQ)	5	92-115	106	9	9
	0.5	5	93-122	102	12	11
CGA 359009	0.05 (LOQ)	5	102-113	107	4	4
	0.5	5	103-128	111	10	9
CGA 363430	0.05 (LOQ)	5	100-125	109	10	9
	0.5	5	91-129	102	15	15
CGA 255548	0.05 (LOQ)	5	90-99	95	4	4
	0.5	5	92-117	101	10	10
SYN 510306	0.05 (LOQ)	5	97-110	102	5	5
	0.5	5	97-130	108	13	12
CGA 294849	0.05 (LOQ)	5	74-88	81	5	6
	0.5	5	77-84	81	3	3
CGA 371075	0.05 (LOQ)	5	68-81	75	5	6
	0.5	5	80-84	82	2	2
CGA 249257	0.05 (LOQ)	5	82-123	92	17	19
	0.5	5	73-98	86	12	14
Confirmatory ion						
Pymetrozine (CGA 215944)	0.05 (LOQ)	5	98-102	100	2	2
	0.5	5	103-129	111	10	9
CGA 215525	0.05 (LOQ)	5	101-111	106	4	3
	0.5	5	92-125	102	13	13
CGA 300407	0.05 (LOQ)	5	71-88	80	7	9
	0.5	5	101-128	108	11	10
CGA 363431	0.05 (LOQ)	5	94-114	103	8	8
	0.5	5	93-122	103	11	11
CGA 359009	0.05 (LOQ)	5	102-112	107	4	4
	0.5	5	103-129	111	11	10
CGA 363430	0.05 (LOQ)	5	102-121	110	8	7
	0.5	5	93-127	102	14	14
CGA 255548	0.05 (LOQ)	5	86-103	94	7	8
	0.5	5	94-114	101	8	8
SYN 510306	0.05 (LOQ)	5	98-108	102	4	4
	0.5	5	98-130	108	13	12
CGA 294849	0.05 (LOQ)	5	74-84	78	4	6
	0.5	5	82-84	83	1	1

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
CGA 371075	0.05 (LOQ)	5	73-85	78	6	7
	0.5	5	79-82	81	1	1
CGA 249257	0.05 (LOQ)	5	77-104	88	10	11
	0.5	5	83-111	95	10	11

Data (uncorrected recovery results, Appendix 4, p. 456) were obtained from pp. 41-46; Tables 3-24, pp. 53-74 of MRID 49673101.

- 1 The surface water (Agvise Lab # 12-156; PASC Sample ID 150104-1; Syngenta Study # TK0048240; Sample ID RIMV00312-001 Surface; pH 7.3, total hardness 27 mg CaCO₃ mg/L, total dissolved solids 58 ppm) and ground water (Agvise Lab # 12-157; PASC Sample ID 150104-2; Syngenta Study # TK0048240; Sample ID RIMV00312-002 Ground; pH 7.5, total hardness 59 CaCO₃ mg/L, total dissolved solids 122 ppm) matrices were characterized (p. 25; Table 1, p. 51; Appendix 3, pp. 442-453). These two matrices were the same as those used in the ECM. Two other matrices were reported, as well: surface water (Agvise Lab # 15-33; PASC Sample ID 150208-1; Syngenta Study # N/A; Sample ID RIMV00115-0001; pH 8.6, total hardness 21 mg CaCO₃ mg/L, total dissolved solids 84 ppm) and ground water (Agvise Lab # 15-34; PASC Sample ID 150208-2; Syngenta Study # N/A; Sample ID RIMV00115-0002; pH 7.7, total hardness 54 CaCO₃ mg/L, total dissolved solids 156 ppm). These two other matrices did not appear to be the matrix samples used in the method validation, based on their Sample ID. All characterization was performed by Agvise Laboratories, Northwood, North Dakota; soil samples were provided by the sponsor.
- 2 Two ion transitions (quantitation (Q) and confirmation (C), respectively) were monitored for each analyte: *m/z* 141.0→42.0 (Q) and *m/z* 141.0→124.9 (C) for CGA 294849; *m/z* 140.1→42.1 (Q) and *m/z* 140.1→67.8 (C) for CGA 371075; *m/z* 217.8→105.0 (Q) and *m/z* 217.8→78.0 (C) for pymetrozine (CGA 215944); *m/z* 129.0→45.0 (Q) and *m/z* 129.0→88.1 (C) for CGA 215525; *m/z* 108.2→80.2 (Q) and *m/z* 108.2→53.2 (C) for CGA 300407; *m/z* 250.1→121.1 (Q) and *m/z* 250.1→103.1 (C) for CGA 363431; *m/z* 234.0→105.2 (Q) and *m/z* 234.0→92.1 (C) for CGA 359009; *m/z* 248.0→121.1 (Q) and *m/z* 248.0→103.1 (C) for CGA 363430; *m/z* 124.0→106.1 (Q) and *m/z* 124.0→78.1 (C) for CGA 255548; *m/z* 234.0→121.3 (Q) and *m/z* 234.0→103.1 (C) for SYN 510306; and *m/z* 114.0→73.1 (Q) and *m/z* 114.1→71.1 (C) for CGA 249257 (pp. 33-39).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD values for pymetrozine, CGA 294849, CGA 215525, CGA 300407, CGA 371075, CGA 363431, CGA 359009, CGA 363430, CGA 255548, CGA 249257 and SYN 510306 in water were 0.05 µg/L (0.05 ppb) and 0.025 ng/mL, respectively (pp. 12, 31 of MRID 49673102; pp. 17, 40, 48; Figures 12-55, pp. 88-109 of MRID 49673101). In the ECM, the LOQ was defined as the lowest level of fortification at which the methodology was validated and was demonstrated to have acceptable mean recovery (70 to 110%) and precision (relative standard deviation of $\leq 20\%$). Additionally, the LOQ response for an analyte peak which should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. Additionally, an estimate of the LOD can be taken as three times background noise. The ECM also noted that the LOD may vary between runs and from instrument to instrument. The reported LOD was based on a 50 µL injection volume. The ILV reported the LOQ and LOD from the ECM without justification.

Table 4a. Method Characteristics

Analyte ¹		Pymetrozine	CGA 215525	CGA 300407	CGA 363431	CGA 359009	CGA 363430
Limit of Quantitation (LOQ)		0.05 µg/L (0.05 ppb)					
Limit of Detection (LOD)		0.025 ng/mL (based on a 50 µL injection volume)					
Linearity (calibration curve r ² and concentration range) ²	ECM	r ² = 0.9944 (Q) r ² = 0.9966 (C)	r ² = 0.9970 (Q) r ² = 0.9904 (C)	r ² = 0.9992 (Q) r ² = 0.9982 (C)	r ² = 0.9880 (Q) r ² = 0.9960 (C)	r ² = 0.9990 (Q) r ² = 0.9982 (C)	r ² = 0.9986 (Q) r ² = 0.9841 (C)
		(0.025-1 ng/mL)					
	ILV	r ² = 0.9930 (Q) r ² = 0.9944 (C)	r ² = 0.9898 (Q) r ² = 0.9978 (C)	r ² = 0.9926 (Q) r ² = 0.9914 (C)	r ² = 0.9950 (Q) r ² = 0.9930 (C)	r ² = 0.9922 (Q) r ² = 0.9918 (C)	r ² = 0.9942 (Q) r ² = 0.9940 (C)
		(0.025-1 ng/mL)					
Repeatable	ECM ³	Yes at LOQ and 10×LOQ					
	ILV ⁴	Yes at LOQ and 10×LOQ					
Reproducible		Yes at LOQ and 10×LOQ					
Specific	ECM	Yes, since matrix interferences in the controls were <20% of the LOQ in the ECM, though significant background noise was observed in the LOQ chromatograms which interfered with peak integration. Some non-uniform peak integration was noted.					
		Yes, matrix interferences were <10% of the LOQ.	The C analyte peak was barely distinguished from the baseline at the LOQ in surface and ground water. ⁵ Significant background noise was observed in the LOQ chromatograms.	The analyte peak was barely distinguished from the baseline at the LOQ in surface water. ⁶ Significant background noise was observed in the LOQ chromatograms.	Significant background noise was observed in the LOQ chromatograms.		
	ILV	Yes, since matrix interferences in the controls were <20% of the LOQ in the ILV, though significant background noise was observed in the LOQ chromatograms which interfered with peak integration. Some non-uniform peak integration was noted.					
		Yes, matrix interferences were <5% of the LOQ.	Yes, no matrix interferences were observed; however, major baseline interference was	Yes, matrix interferences were <20% of the LOQ.	Yes, no matrix interferences were observed.	Yes, matrix interferences were <5% of the LOQ.	Yes, no matrix interferences were observed.

			noted in the C chromatogram at the LOQ in ground water. ⁷				
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Data were obtained from pp. 12, 31; Table 2, pp. 37-47 (recovery data); Figures 11-21, pp. 59-89 (calibration chromatograms); Figures 22-32, pp. 91-112 (calibration curves and coefficients); Figures 33-65, pp. 114-191 (chromatograms) of MRID 49673102; pp. 17, 31, 41-46; Tables 3-24, pp. 53-74 (recovery data); Figures 56-77, pp. 110-120 (calibration curves and coefficients); Figures 78-231, pp. 121-197 (chromatograms) of MRID 49673101; DER Attachment 2. Q = quantitation ion; C = confirmatory ion.

1 Pymetrozine = (E)-4,5-Dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one; CGA 215525 = 4-Amino-6-methyl-2,5-dihydro-1,2,4-triazin-3-one; CGA 300407 = 3-Pyridinecarbaldehyde; CGA 363431 = 5-Hydroxy-6-methyl-4- {[1-(6-oxo-1,6-dihydro-pyridin-3-yl)-meth-(E)-ylidene]-amino}-4,5-duhydro-2H-[1,2,4]triazin-3-one; CGA 359009 = 4,5-Dihydro-5-hydroxy-6-methyl-4-[(3-pyridinylmethylene)amino]-1,2,4-triazine-3-(2H)-one; CGA 363430 = 6-Methyl-4- {[1-(6-oxo-1,6-dihydro-pyridin-3-yl)-meth-(E)-ylidene]-amino}-2H-[1,2,4]triazine-3,5-dione.

2 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 22-32, pp. 91-112 of MRID 49673102; Figures 56-77, pp. 110-120 of MRID 49673101; DER Attachment 2). Matrix-matched calibration standards were used in the ECM and ILV, but only one set of calibration curves was provided (p. 31; of MRID 49673102; p. 31 of MRID 49673101)

3 In the ECM, the surface (local lake) water (pH 7.3, total hardness 27 CaCO₃ mg/L) and ground (local well) water (pH 7.5, total hardness 59 mg CaCO₃ mg/L) matrices were partially characterized (Table 1, p. 36 of MRID 49673102). The soil sources were not further specified.

4 In the ILV, the surface water (Agvise Lab # 12-156; PASC Sample ID 150104-1; Syngenta Study # TK0048240; Sample ID RIMV00312-001 Surface; pH 7.3, total hardness 27 mg CaCO₃ mg/L, total dissolved solids 58 ppm) and ground water (Agvise Lab # 12-157; PASC Sample ID 150104-2; Syngenta Study # TK0048240; Sample ID RIMV00312-002 Ground; pH 7.5, total hardness 59 CaCO₃ mg/L, total dissolved solids 122 ppm) matrices were characterized (p. 25; Table 1, p. 51; Appendix 3, pp. 442-453 of MRID 49673101). These two matrices were the same as those used in the ECM; ILV soil samples were provided by the sponsor.

5 Based on Figure 35, pp. 121-122 and Figure 46, pp. 154-155 of MRID 49673102.

6 Based on Figure 36, p. 124 of MRID 49673102.

7 Based on Figure 121, p. 142 of MRID 49673101.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

Linearity is satisfactory when $r^2 \geq 0.995$.

Table 4b. Method Characteristics

Analyte ¹		CGA 255548	SYN 510306	CGA 294849	CGA 371075	CGA 249257
Limit of Quantitation (LOQ)		0.05 µg/L (0.05 ppb)				
Limit of Detection (LOD)		0.025 ng/mL (based on a 50 µL injection volume)				
Linearity (calibration curve r ² and concentration range) ²	ECM	r ² = 0.9998 (Q) r ² = 0.9984 (C)	r ² = 0.9944 (Q) r ² = 0.9855 (C)	r ² = 0.9994 (Q) r ² = 0.9988 (C)	r ² = 0.9996 (Q) r ² = 0.9998 (C)	r ² = 0.9998 (Q) r ² = 0.9996 (C)
		(0.015-2.0 µg/L)				
	ILV	r ² = 0.9934 (Q) r ² = 0.9914 (C)	r ² = 0.9841 (Q) r ² = 0.9894 (C)	r ² = 0.9841 (Q) r ² = 0.9894 (C)	r ² = 0.9847 (Q) r ² = 0.9819 (C)	r ² = 0.9998 (Q) r ² = 0.9996 (C)
		(0.015-2.0 µg/L)				
Repeatable	ECM ³	Yes at LOQ and 10×LOQ				
	ILV ⁴	Yes at LOQ and 10×LOQ				
Specific	ECM	Yes, since matrix interferences in the controls were <5% of the LOQ in the ECM, though significant background noise was observed in the LOQ chromatograms which interfered with peak integration. Some non-uniform peak integration was noted.				
		The analyte peak was barely distinguished from the baseline at the LOQ in surface water. ⁵ Significant background noise was observed in the LOQ chromatograms.	Significant background noise was observed in the LOQ chromatograms.			The baseline was highly irregular in surface and ground water at the LOQ/10×LOQ. ⁶ Significant background noise was observed in the LOQ chromatograms.
	ILV	Yes, though significant background noise was observed in the LOQ chromatograms which interfered with peak integration. Some non-uniform peak integration was noted.				
		Yes, no matrix interferences were observed.	Yes, matrix interferences were <10% of the LOQ.	Yes, no matrix interferences were observed; however, major baseline interference was noted in the LOQ chromatogram in surface water. ⁷	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed; however, the baseline was highly irregular in surface and ground water at the LOQ/10×LOQ. ⁸

Data were obtained from pp. 12, 31; Table 2, pp. 37-47 (recovery data); Figures 11-21, pp. 59-89 (calibration chromatograms); Figures 22-32, pp. 91-112 (calibration curves and coefficients); Figures 33-65, pp. 114-191 (chromatograms) of MRID 49673102; pp. 17, 31, 41-46; Tables 3-24, pp. 53-74 (recovery data);

Figures 56-77, pp. 110-120 (calibration curves and coefficients); Figures 78-231, pp. 121-197 (chromatograms) of MRID 49673101; DER Attachment 2. Q = quantitation ion; C = confirmatory ion.

1 CGA 255548 = 6-Oxo-1,6-dihydro-pyridine-3-carbaldehyde; SYN 510306 = 6-Methyl-4-[(6-oxo-1,6-dihydro-pyridin-3-ylmethylene)-amino]-4,5-dihydro-2H-[1,2,4]triazin-3-one; CGA 294849 = 4-Amino-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione; CGA 371075 = 4,6-Dimethyl-2H-1,2,4-triazine-3,5-dione; CGA 249257 = 6-Methyl-4,5-dihydro-2H-1,2,4-triazin-3-one.

2 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 22-32, pp. 91-112 of MRID 49673102; Figures 56-77, pp. 110-120 of MRID 49673101; DER Attachment 2).

3 In the ECM, the surface (local lake) water (pH 7.3, total hardness 27 CaCO₃ mg/L) and ground (local well) water (pH 7.5, total hardness 59 mg CaCO₃ mg/L) matrices were partially characterized (Table 1, p. 36 of MRID 49673102). The soil sources were not further specified.

4 In the ILV, the surface water (Agvise Lab # 12-156; PASC Sample ID 150104-1; Syngenta Study # TK0048240; Sample ID RIMV00312-001 Surface; pH 7.3, total hardness 27 mg CaCO₃ mg/L, total dissolved solids 58 ppm) and ground water (Agvise Lab # 12-157; PASC Sample ID 150104-2; Syngenta Study # TK0048240; Sample ID RIMV00312-002 Ground; pH 7.5, total hardness 59 CaCO₃ mg/L, total dissolved solids 122 ppm) matrices were characterized (p. 25; Table 1, p. 51; Appendix 3, pp. 442-453 of MRID 49673101). These two matrices were the same as those used in the ECM; ILV soil samples were provided by the sponsor.

5 Based on Figure 40, p. 136 of MRID 49673102.

6 Based on Figure 43, p. 145 and Figure 54, pp. 178-179 of MRID 49673102.

7 Based on Figures 168-169, p. 166 of MRID 49673101.

8 Based on Figure 162, p. 163, Figure 164, p. 164, and Figures 228-229, p. 196 of MRID 49673101.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies

1. The ECM method was validated by the ILV with multiple modifications to sample preparation, injection volume, mobile phase gradient (for CGA 249257 in ground water only) and mass spectrometer parameters (pp. 31-39; Appendix 5, pp. 457-467 of MRID 49673101). These ILV modifications were not included in an amended final ECM report. Based on the communications, the ILV modifications to the ECM method for CGA 249257 were necessary to generate a successful trial. Even between the two water matrices, surface and ground, two different sets of ILV modifications were required to generate acceptable results.
2. For the calibration curves of the ILV, the following correlation coefficients were unsatisfactory ($r^2 < 0.995$): pymetrozine [$r^2 = 0.9930$ (Q), 0.9944 (C)]; CGA 215525 [$r^2 = 0.9898$ (Q)]; CGA 300407 [$r^2 = 0.9926$ (Q), 0.9914 (C)]; CGA 363431 [$r^2 = 0.9930$ (C)]; CGA 359009 [$r^2 = 0.9922$ (Q), 0.9918 (C)]; CGA 363430 [$r^2 = 0.9942$ (Q), 0.9940 (C)]; CGA 255548 [$r^2 = 0.9934$ (Q), 0.9914 (C)]; SYN 510306 [$r^2 = 0.9841$ (Q), 0.9894 (C)]; CGA 294849 [$r^2 = 0.9841$ (Q), 0.9894 (C)]; CGA 371075 [$r^2 = 0.9847$ (Q), $r^2 = 0.9819$ (C)]; Figures 56-77, pp. 110-120 of MRID 49673101; DER Attachment 2].

For the calibration curves of the ECM, the following correlation coefficients were unsatisfactory ($r^2 < 0.995$): pymetrozine [$r^2 = 0.9944$ (Q)]; CGA 215525 [$r^2 = 0.9904$ (C)]; CGA 363431 [$r^2 = 0.9880$ (Q)]; CGA 363430 [$r^2 = 0.9841$ (C)]; and SYN 510306 [$r^2 = 0.9944$ (Q), 0.9855 (C)]; Figures 22-32, pp. 91-112 of MRID 49673102; DER Attachment 2].

The reviewer noted that only one set of calibration curves were provided in the ECM and ILV even though matrix-matched standards were used (p. 47 of MRID 49673102; pp. 30-31 of MRID 49673101).

3. In the ECM, significant matrix effects were observed ($>20\%$); matrix-matched calibration standards were used (pp. 31, 47; Table 3, p. 48 of MRID 49673102).
4. The specificity of the method was considered satisfactory since matrix interferences in the controls were $<20\%$ of the LOQ in the ECM and ILV, $<50\%$ of the LOD (based on peak areas); however, the reviewer noted significant baseline noise in most of the LOQ chromatograms of the ECM and ILV (Figures 33-65, pp. 114-191 of MRID 49673102; Figures 78-231, pp. 121-197 of MRID 49673101). In many of these LOQ chromatograms, the baseline noise around the LOQ peak was approximately 20-50% of the height of the LOQ peak. In these cases, the high height of the baseline noise caused difficulty with uniform peak integration. Additionally, the baseline was very irregular in many chromatograms, especially those of CGA 249257. Overall, the retention time of the analyte was the best indication of analyte peak position.
5. The reviewer determined that the ground and surface water matrices of the ECM were the same as those of the ILV based on the water characteristics and the fact that the ILV

water samples were provided by the sponsor (Table 1, p. 36 of MRID 49673102; p. 25; Table 1, p. 51; Appendix 3, pp. 442-453 of MRID 49673101).

In the ILV, two additional matrices were reported: one surface water and one groundwater (p. 25; Table 1, p. 51; Appendix 3, pp. 442-453 of MRID 49673101). These two other matrices did not appear to be the matrix samples or additional matrix samples used in the method validation, based on their Sample ID. Only one type of ground and surface water matrices were used in the ILV.

6. The r^2 values for the calibration curves (Figures 56-77, pp. 110-120 of MRID 49673101) are unconventionally reported as r .
7. The reviewer noted the following significant typographical error in the ECM: the calibration curves for CGA 294849 were incorrec~~t~~ed titled as “Representative Calibration Curves for CGA2948~~9~~28” (Figure 31, pp. 109-110 of MRID 49673102).

V. Reviewer’s Comments

1. Although the mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%), many individual recovery ranges were outside this range, especially in the groundwater matrix of the ILV.
2. Communications between the ILV and study sponsor were provided (p. 47; Appendix 5, pp. 457-467 of MRID 49673101). The communications involved ECM study clarification, relaying ILV study results, requesting approval of ILV modifications to the ECM and detailed list of ILV modifications.
3. It was reported for the ILV that one batch of 13 samples required one working day to complete (p. 47 of MRID 49673101). Instrument analysis of water samples was performed overnight.
4. In the ECM, the stability of the stock solutions and final extracts was investigated (pp. 32, 47; Tables 4-5, pp. 49-50 of MRID 49673102). Final extracts of CGA 294849, CGA 371075 and CGA 249257 in groundwater were stored at 4° and found to be stable for up to 9 days. All of the other analytes in groundwater were found to be unstable in these storage conditions. In surface water, residues of CGA 363431 and CGA 363430 were unstable (>15%) in final sample fractions at 4°C for 8 days. It was recommended that the final extracts are injected into the LC/MS/MS system immediately. Stock solutions of CGA 363431, CGA 363430 and CGA 294849 were stored at 4° and found to be unstable (>15%) after 14 days (freezing conditions for CGA 300407). It was recommended that fresh stock solutions are prepared every 14 days and stored in freezing conditions.
5. While the r^2 values of the calibration curves are unsatisfactory, increasing the lower end of the calibration curve and removing the data points containing high baseline noise will help improve the linear fit and improve the r^2 values to acceptable levels.

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.

DER Attachment 1: Chemical Names and Structures



101103_49673101+_8
50.6100_Calculations.x

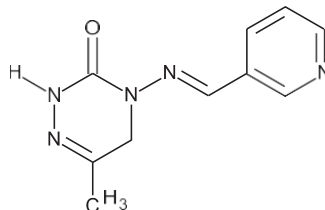
Pymetrozine (CGA 215944; CSAA202913)

IUPAC Name: (E)-4,5-Dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one

CAS Name: 4,5-Dihydro-6-methyl-4-[(E)-(3-pyridinylmethylene)amino]-1,2,4-triazin-3(2H)-one

CAS Number: 123312-89-0

SMILES String: O=C1NN=C(C)CN1N=Cc2ccnc2



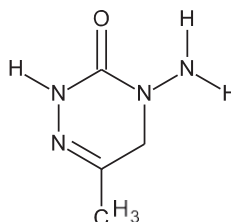
CGA 215525 (CSAA202517)

IUPAC Name: 4-Amino-6-methyl-2,5-dihydro-1,2,4-triazin-3-one

CAS Name: Not reported

CAS Number: Not found

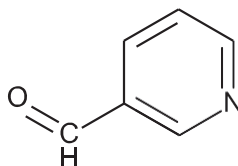
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CGA 300407 (CSAA086506; M3)

IUPAC Name: 3-Pyridinecarboxaldehyde
Pyridine-3-carbaldehyde

CAS Name: Not reported
CAS Number: Not found
SMILES String: O=Cc1ccncc1



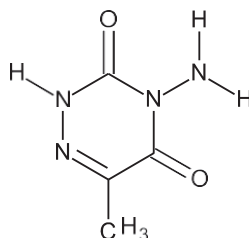
CGA 294849 (CSAA377032; Metabolite B)

IUPAC Name: 4-Amino-6-methyl-1,2,4-triazine-3,5(2H,4H)-dione
4-Amino-6-methyl-2H-1,2,4-triazine-3,5-dione

CAS Name: Not reported

CAS Number: Not found

SMILES String: CC1=NNC(=O)N(N)C1=O

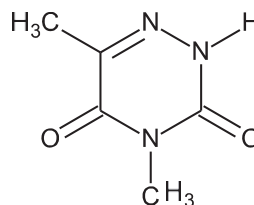
**CGA 371075 (CSAA447511; Metabolite C)**

IUPAC Name: 4,6-Dimethyl-2H-1,2,4-triazine-3,5-dione
4,6-Dimethyl-2H-[1,2,4]triazine-3,5-dione

CAS Name: Not reported

CAS Number: Not found

SMILES String: CN1C(=O)NN=C(C)C1=O

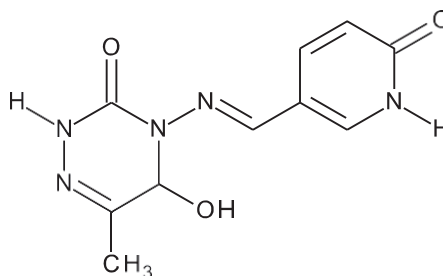
**CGA 363431 (CSAA452413; CSAA444591; MB5)**

IUPAC Name: 5-Hydroxy-6-methyl-4-[(6-oxo-1,6-dihydro-pyridine-3-ylmethylene)-amino]-4,5-dihydro-2H-[1,2,4]triazine-3-one
5-Hydroxy-6-methyl-4- {[1-(6-oxo-1,6-dihydro-pyridin-3-yl)-meth-(E)-ylidene]-amino} -4,5-duhydro-2H-[1,2,4]triazin-3-one

CAS Name: Not reported

CAS Number: Not found

SMILES String: CC1=NNC(=O)N(\N=C\C2=CN(C(=O)C=C2)C1O



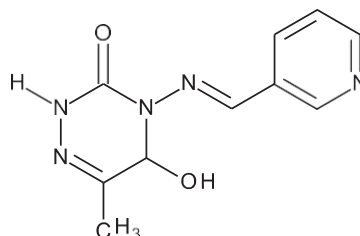
CGA 359009 (CSAA441607; Metabolite D, M4, Metabolite 2U)

IUPAC Name: 4,5-Dihydro-5-hydroxy-6-methyl-4-[(3-pyridinylmethylene)amino]-1,2,4-triazine-3-(2H)-one
5-Hydroxy-6-methyl-4-[(E)-3-pyridylmethyleneamino]-2,5-dihydro-1,2,4-triazin-3-one

CAS Name: Not reported

CAS Number: Not found

SMILES String: CC1=NNC(=O)N(\N=C\c2ccnc2)C1O

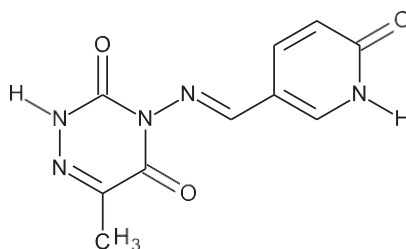
**CGA 363430 (CSAA444590; MB4)**

IUPAC Name: 6-Methyl-4-[[1-(6-oxo-1,6-dihydro-pyridin-3-yl)-meth-(E)-ylidene]-amino]-2H-[1,2,4]triazine-3,5-dione

CAS Name: Not reported

CAS Number: Not found

SMILES String: CC1=NNC(=O)N(\N=C\C2=CNC(=O)C=C2)C1=O

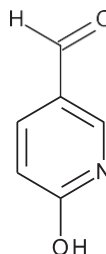
**CGA 255548 (CSAA241313)**

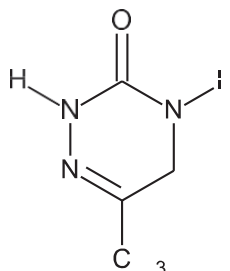
IUPAC Name: 6-Oxo-1,6-dihydro-pyridine-3-carbaldehyde

CAS Name: Not reported

CAS Number: Not found

SMILES String: Oc1ccc(C=O)cn1



CGA 249257 (CSAA235363)**IUPAC Name:** 6-Methyl-4,5-dihydro-2H-1,2,4-triazin-3-one**CAS Name:** Not reported**CAS Number:** Not found**SMILES String:** CC1=NNC(=O)NC1**SYN 510306 (CSCC213345)****IUPAC Name:** 6-Methyl-4-[(6-oxo-1,6-dihydro-pyridin-3-ylmethylene)-amino]-4,5-dihydro-2H-[1,2,4]triazin-3-one**CAS Name:** Not reported**CAS Number:** Not found**SMILES String:** CC1=NNC(=O)N(C1)\N=C\C2=CN(C(=O)C=C2)