STUDY TITLE

Independent Laboratory Validation of Method Validation for Determination of Nitrapyrin and 6-CPA in Water

DATA REQUIREMENTS

U.S. Environmental Protection Agency: 40 CFR Part 160

Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes., SANTE/2020/12830 rev. 1., 24 February 2021

Ecological Effects Test Guidelines, OCSPP 850.6100 Environmental Chemistry Methods and Associated Independent Laboratory Validation, U.S. Environmental Protection Agency, January 11, 2012

EXPERIMENTAL

Specimen Procurement

Bulk control samples of surface water, ground water, and drinking water were sourced locally by EPL BAS. Water samples were characterized by AGVISE Laboratories, 804 Highway 15 West, Northwood, ND 58267, USA as specified in OCSPP 850.6100. Characterization included pH, calcium, magnesium, turbidity, conductivity, hardness, total suspended solids, alkalinity, total organic carbon, and dissolved organic carbon. The bulk control samples were stored refrigerated (ca. 4 °C) prior to use in the laboratory and after sample aliquots were removed.

Reference Items

The certificate of analysis for each reference item can be found in Appendix B. The analytical grade materials were supplied by the Sponsor and maintained ambient.

Standard stock solutions, calibration standard solutions and fortification solutions were prepared as described in the analytical methods. Full details of these materials are included in the raw data package for the study along with details of the preparation of all analytical and fortification standards prepared from the primary reference items. The reference items will be retained until expiry and then disposed of following relevant disposal standard operating preocedures (SOPs) with the approval of the Sponsor Representative.

The following fortification scheme was used:

Matrix	Reference Item	Reagent Blank	Untreated Control	Fortification Level 0.05 µg/L (LOQ)*	Fortification Level 0.5 µg/L (10x LOQ)
Surface Water	Nitrapyrin	1	2	5	5
	6-CPA	1	2	5	5
Ground Water	Nitrapyrin	1	2	5	5
	6-CPA	1	2	5	5
Drinking Water	Nitrapyrin	1	2	5	5
	6-CPA	1	2	5	5

*LOQ – Limit of quantitation

Nitrapyrin Method

Specimens were assayed according to the method appearing in Appendix 1 of report 220060. The detailed analytical procedure appears in Appendix C.

Calibration Regression Equation: y = 429.268372 * x + 43.184875
Amount Found (ng/mL) = (1190 – 43.184875) / 429.268372 = 2.672

Note: The hand calculated value (2.672) differs slightly from the value obtained in the Excel calculation (2.671) due to rounding differences.

The concentration found in each extract was determined using the formula:

µg/L Found =

$$\frac{\textit{Amount Found (ng/mL)} * \textit{Final Volume (mL)}}{\textit{Sample Volume (mL)}}$$

Example: Set T004, Surface Water
Sample ID: 3454-S001-S11, LOQ Fortification
Amount Found: 2.671 ng/mL
Fianl Volume: 2 mL
Sample Volume: 100 mL
µg/L Found = 2.671 * 2 / 100 = 0.0534

Recovery of fortified specimens was calculated as follows:

Fortification Level (µg/L) =

$$\frac{\textit{Spiking Solution Concentration (ng/mL)} * \textit{Volume Spiking Solution (mL)}}{\textit{Sample Volume (mL)}}$$

Example: Set T004, Surface Water
Sample ID: 3454-S001-S11, LOQ Fortification
Spiking Solution Concentration: 10.000 ng/mL
Volume Spiking Solution: 0.500 mL
Sample Volume: 100 mL
Fortification Level (µg/L) = 10.000 * 0.500 / 100 = 0.050

Recovery (%) =

*(µg/L Found Fortified Sample - µg/L Unfortified Control) / Fortification Level (µg/L) * 100*

Example: Set T004, Surface Water
Sample ID: 3454-S001-S11, LOQ Fortification
µg/L Found: 0.0534
Fortification Level: 0.050 µg/L
Recovery (%) = (0.0534 / 0.050) *100 = 107

6-CPA Method

Specimens were assayed according to the method appearing in Appendix 2 of report 220060. The detailed analytical procedure appears in Appendix D.

Method Principle:

20 mL of water sample was acidified with 2 mL of 1N hydrochloric acid. The entire sample was passed through a C18 SPE cartridge that was preconditioned with 5 mL of 0.1N hydrochloric acid. The column was washed with 2 mL of 0.1N hydrochloric acid and dried under vacuum for 45 minutes. 6-CPA was eluted from the column with 5 mL of acetonitrile. The acetonitrile was evaporated to dryness under a stream of nitrogen set to 35 °C, and the residue redissolved with 0.5 mL of methanol:deionized (DI) water (25:75, v/v) for high performance liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS). Two MRM transitions were used; m/z 156>112 was the primary or quantitative transition (Q) and m/z 158>114 was the secondary or confirmatory transition (C). Instrumental analysis was conducted using an Agilent 1290 HPLC system with an AB SCIEX 6500 triple quadrupole mass spectrometer detector. Some instrument parameters differed slightly than those appearing in the analytical method due to differences in make and model of the systems used in the initial validation study and this ILV study.

Example Calculations*:*

LC-MS/MS calibration curves were generated using linear regression analysis of the peak area (y-axis) vs. concentration in ng/mL (x-axis) for 6-CPA standard solutions analyzed with each set of samples. The calibration standard concentrations encompassed a range from 0.4 to 40 ng/mL. Calibration standards were prepared in neat solvent (methanol:DI water, 25:75 v/v). The amount found (ng/mL) of analyte was calculated from the linear regression equation of the calibration curve. A weighting function of 1/x was used. The origin was excluded from the regression equation.

$y = mx + b$

Where:

y = Peak area
m = Slope of linear regression equation
x = Concentration in ng/mL
b = y-Intercept of linear regression equation

The linear regression equation was used to solve for x, which correlates to amount found in ng/mL.

Example: Set T001, Surface Water
Sample ID: 3454-S001-S1, LOQ Fortification
Peak Area (m/z 156>112 (Q) transition): 109087
Calibration Regression Equation: y = 57432.9 * x + 8100.82053

Amount Found (ng/mL) =

((109807 – 8100.82053) / 57432.9 = 1.758

The concentration found in each sample was determined using the formula:

µg/L Found =

$$\frac{\textit{Amount Found (ng/mL)} * \textit{Final Volume (mL)}}{\textit{Sample Volume (mL)}}$$

Example: Set T001, Surface Water
Sample ID: 3454-S001-S1, LOQ Fortification
Amount Found: 1.758 ng/mL
Final Volume: 0.5 mL
Sample Volume: 20 mL

µg/L Found = (1.758 * 0.5) / 20 = 0.0440

Recovery of fortified specimens was calculated as follows:

Fortification Level (µg/L) =

$$\frac{\textit{Spiking Solution Concentration (ng/mL)} * \textit{Volume Spiking Solution (mL)}}{\textit{Sample Volume (mL)}}$$

Example: Set T001, Surface Water
Sample ID: 3454-S001-S1, LOQ Fortification
Spiking Solution Concentration: 101.000 ng/mL
Volume Spiking Solution: 0.010 mL
Sample Volume: 20 mL
Fortification Level (ppm) = 101.000 * 0.010 / 20 = 0.0505

Recovery (%) =

*(µg/L Found Fortified Sample / Fortification Level (µg/L) * 100*

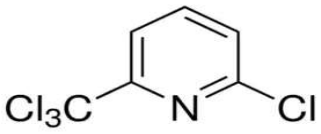
Example: Set T001, Surface Water
Sample ID: 3454-S001-S1, LOQ Fortification
µg/L Found: 0.0440
Fortification Level: 0.0505 µg/L
Recovery (%) = (0.0440) / 0.0505) *100 = 87.0

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries for a fortification level was calculated using the "STDEV" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. The % relative

Table 1. Nitrapyrin Structure

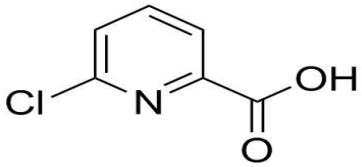
Common name:	Nitrapyrin
Chemical name (IUPAC):	2-Chloro-6-(trichloromethyl) pyridine
CAS-Registry-No.:	1929-82-4
Chemical structure:	



Molecular formula:	$C_6H_3Cl_4N$
TSN Number:	TSN003679-0002
Batch/Lot reference:	V43-037266-95
Purity:	99.6%
Expire date:	31/Jul/2030

Table 2. 6-Chloropicolinic Acid Structure

Common name:	6-Chloropicolinic acid
Chemical name (IUPAC):	6-Chloropyridine-2-carboxylic acid
CAS-Registry-No.:	4684-84-0
Chemical structure:	



Molecular formula:	$C_6H_4ClNO_2$
TSN Number:	AGR029021
Batch/Lot reference:	188
Purity:	99%
Expire date:	08/Jul/2027