Analytical method for flonicamid (IKI-220) and its metabolites TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM in water

Reports: ECM 1: EPA MRID No.: MRID 45854816. Wais, A. 2002. Validation of the Residue Analytical Method for IKI-220 and Metabolites (TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM) in Surface Water and Drinking Water. Report prepared by RCC Ltd, Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland; sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan; and submitted by ISK Biosciences Corporation, Concord, Ohio; 75 pages (including 3 front pages). Document No.: 834131. Final report issued August 22, 2002.

ECM 2 (Supplemental Data for ECM 1): EPA MRID No.: MRID 47318701. Wais, A. 2007. Response to EPA Review and Submission of Supplemental Data to Report: Validation of the Residue Analytical Method for IKI-220 and Metabolites (TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM) in Surface Water and Drinking Water (MRID#45854816). Report prepared by RCC Ltd, Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland; sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan; and submitted by ISK Biosciences Corporation, Concord, Ohio; 8 pages (including 3 front pages). Document No.: RCC 834131. Final report issued December 20, 2007.

ILV: EPA MRID No.: MRID 50143203. Schoenau, E.A. 2016. Independent Laboratory Validation of Ishihara Sangyo Kaisha (ISK) Residue Analytical Method for the Determination of IKI-220 and Metabolites (TFNA, TFNG, TFNA-AM, and TFNG-AM) in Surface Water and Drinking Water (Document Number: 834131). Report prepared by Golden Pacific Laboratories, LLC (GPL), Fresno, California; sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan; and submitted by ISK Biosciences Corporation, Concord, Ohio; 226 pages. GPL Study No.: 150589. Final report issued December 22, 2016.

Document No.: MRIDs 45854816 & 47318701 & 50143203

Guideline: 850.6100

Statements: ECM 1: The study was conducted in compliance with USEPA FIFRA (40 CFR Part 160) and Swiss Good Laboratory Practice (GLP) standards (pp. 1C, 2-3 of MRID 45854816). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 1B-1C, 2-4). The statement of authenticity was not included.
ECM 2: The study was conducted in compliance with USEPA FIFRA GLP standards (p. 1C of MRID 47318701). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 1B-1C, 3). The statement of authenticity was not included.
ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (p. 3 of MRID 50143203). 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 due to the discrepancies

identified in the Section IV. Method Deficiencies and Reviewer's Comments. However, an updated ILV should be submitted for TFNA-OH following the method and amendments provided to determine if it can be independently validated.

PC Code:	128016	
EFED Final Reviewer:	Jessica Murillo, PhD	Signature: JESSICA MURILLO Digitally signed by JESSICA MURILLO Date: 2025.04.22 16:01:58 -04'00' Date: 04/22/2025
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature: Lisa Muto Date: 03/5/2019
	Mary Samuel, M.S., Environmental Scientist	Signature: Marysamuel Date: 03/5/2019

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, ISK Biosciences Corporation Document No. 834131, is designed for the quantitative determination of flonicamid (IKI-220) and its metabolites TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM in water at the stated LOQ of 0.1 µg/L. The LOQ is less than the lowest toxicological level of concern in water for all six analytes. The ECM and ILV used characterized drinking and surface waters. **TFNA-OH was not included as an analyte in the ILV**; therefore, the reproducibility of the method for TFNA-OH could not be determined since only one set of performance data was submitted, that of the ECM. The ILV validated the ECM for five analytes (flonicamid IKI-220, TFNA, TFNG, TFNA-AM, and TFNG-AM) in the second trial for drinking water and in the third trial for surface water. The ILV noted that failed trials were due to cross-contamination at the rotary evaporator stations and to the pH being >4.3 and suggested that the method be modified to emphasize thorough cleaning of all equipment and that all sample pH must be suitably adjusted rather than providing a blanket adjustment step for all samples. The ILV also reported that sample dilutions were necessary to combat signal enhancement and obtain acceptable results (solvent-based calibration standards were used); however, for TFNG, the ionization source had to be changed from APCI to ESI to eliminate signal enhancement. Submitted ILV and ECM data pertaining to precision, repeatability, reproducibility, linearity, and specificity was acceptable at the LOQ and 10×LOQ for five analytes in both waters, this excludes reproducibility of TFNA-OH in the ILV (p. 42 of MRID 50143203), and ECM linearity for one of two calibration curves of TFNG (p. 32 of MRID 45854816). Submitted ECM data pertaining to precision, repeatability, linearity, and specificity of TFNA-OH was acceptable at the LOQ and 10×LOQ in both waters, except for linearity for one of two calibration curves (p. 32 of MRID 45854816).

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						
Flonicamid (IKI-220)	MRIDs 45854816[2] & 47318701[3]	MRID 50143203[4]		Water	22/08/2002	ISK Biosciences Corporation	LC/MS/MS	0.1 µg/L
TFNA								
TFNG								
TFNA-AM								
TFNA-OH[4]								
TFNG-AM								

1 Flonicamid = N-cyanomethyl-4-trifluoromethyl-nicotinamide; TFNA = 4-Trifluoromethylnicotinic acid; TFNG = N-(4-trifluoromethylnicotinoyl)glycine; TFNA-AM = 4-Trifluoromethylnicotinamide; TFNA-OH = 6-Hydroxy-4-trifluoromethylnicotinic acid; and TFNG-AM = N-(4-trifluoromethylnicotinoyl)glycinamide.

2 In the ECM 1, the drinking water (pH 7.42, hardness 23° DH, dissolved organic carbon 2.1 mg/L, silt content 40 mg/L) was collected from a tap at RCC; the surface water (pH 8.12, hardness 26° DH, dissolved organic carbon 2.4 mg/L, silt content 20 mg/L) was sampled from Ergolz in Lausen, Switzerland (p. 17 of MRID 45854816).

3 ECM 2 MRID 47318701 contained supplemental information for ECM 1 MRID 45854816. The supplemental information contained method procedure details and no additional data.

4 In the ILV, the drinking water (pH not reported, hardness 122 mg/L $CaCO_3$, total dissolved solids 234 mg/L) was obtained from a faucet (municipal supply) located at GPL; surface water (pH 7.1, hardness 9 mg/L $CaCO_3$, total dissolved solids 94 mg/L) was obtained from a point on the San Joaquin River near Nees and Palm Avenues in Fresno, California (pp. 13, 22-24; Appendix C, pp. 101-113 of MRID 50143203). The surface water was characterized by Agvise Laboratories, Northwood, North Dakota. Characterization data for the drinking water was provided by the City of Fresno Water Division. **TFNA-OH was not included in the ILV**.

I. Principle of the Method

Water samples (1000 mL) were fortified (0.1 mL of either 1.0 µg/mL or 10.0 µg/mL solutions) and acidified to pH <3 with 2 mL of hydrochloric acid (32%; pp. 18, 21 of MRD 45854816; p. 5 of MRID 47318701). An Isolute ENV+ (500 mg/6 mL) solid phase extraction (SPE) column was pre-conditioned sequentially with 5 mL each of dichloromethane, methanol, distilled water, and hydrochloric acid (*ca*. 0.1M). The ENV+ column was filled with 5 mL of hydrochloric acid (*ca*. 0.1M), and acidified sample was applied to and passed through the column (rate <10 mL/minute). After the column was dried via vacuum for about 10 seconds, the analytes were eluted with three aliquots of 5 mL of methanol. The combined eluates were reduced to dryness under vacuum at *ca*. 50-60°C using a rotary evaporator. The residue was reconstituted in 10 mL of ethanol and evaporated again to dryness. The residue was reconstituted in 2.0 mL of water:acetonitrile (9:1, v:v) using an ultrasonic bath.

Analytes were identified and quantified by LC/MS using a Merck-L-7200 system coupled to a Finnigan MAT TSQ mass spectrometer (pp. 22-23 of MRID 45854816). The following conditions were employed: Phenomenex Luna C8 column (3.0 × 150 mm, 3 µm; column temperature 40°C) with Phenomenex Security Guard C8 column (3.0 × 4 mm) eluted with a gradient mobile phase of (A) 0.05% formic acid in water and (B) methanol [mobile gradient phase of percent A:B (v:v) at 0-2 min. 90:10, 4 min. 78:22, 12 min. 30:70, 15-20 min. 10:90, 20.1-28 min. 90:10] and injection volume of 40 µL; and positive APCI ionization SRM scan mode with 500°C vaporizer temperature. Analytes were identified using one ion transition: *m/z* 229.8→203.1 for flonicamid (IKI-220), *m/z* 192.1→147.9 for TFNA, *m/z* 249.0→202.9 for TFNG, *m/z* 191.2→148.1 for TFNA-AM, *m/z* 208.1→145.9 for TFNA-OH, and *m/z* 248.0→174.0 for TFNG-AM. Expected retention times were *ca*. 9.5, 11.6, 9.3, 7.5, 10.0, and 7.1 minutes for flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM, respectively.

The ILV performed the ECM method as written, except for sample acidification was achieved using 2.5 mL and 2 mL of hydrochloric acid solution (*ca*. 0.1M) for the surface and drinking water samples, respectively, the use of concentrated hydrochloric acid to prep the ENV+ SPE column, the use of ESI instead of APCI for TFNG analysis, additional sample dilutions prior to analysis, and insignificant modifications to the analytical equipment and parameters (pp. 24-31; Appendix B, pp. 97-100 of MRID 50143203). Additionally, TFNA-OH was not included as an analyte in the final validation trial for surface water (ILV trial 3) and drinking water (ILV trial 2). Samples were analyzed for analytes using an AB Sciex API4000 LC-MS/MS with Shimadzu LC-20AD XR HPLC. The LC/MS/MS parameters were the same as those of the ECM, except that injection volume was 25 µL and TEM was 550°C. Additionally, analytes IKI-220, TFNA, TFNA-AM, and TFNG-AM were analyzed using an APCI source, whereas TFNG was analyzed using an ESI source. Using APCI, analytes were identified using one ion transition: *m/z* 229.8→203.1 for flonicamid (IKI-220), *m/z* 191.6→148.1 for TFNA, *m/z* 248.8→203.0 for TFNG (not used), *m/z* 190.6→148.2 for TFNA-AM, and *m/z* 247.7→174.0 for TFNG-AM. These were similar to those of the ECM. Using ESI, TFNG was identified using two ion transitions: *m/z* 249.1→203.0 for primary and *m/z* 249.1→148.0 for confirmatory; only the primary ion transition was quantified. Expected retention times were *ca*. 8.2, 9.7, 8.0, 6.7, and 6.3 minutes for flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, and TFNG-AM, respectively. The ILV noted that failed trials were due to cross-contamination which was occurring at the rotary evaporator stations (p. 33). For the successful method validation attempts, the rotary evaporator stations were thoroughly cleaned prior to the second evaporation to prevent cross-contamination. The ILV noted that the materials used for SPE

(i.e., reservoirs, valve, etc.) must not be degraded by dichloromethane or concentrated hydrochloric acid. The ILV also noted that failed trials were due to the pH being >4.3 and suggested that the method be modified to emphasize that all sample pH must be suitably adjusted rather than providing a blanket adjustment step for all samples (pp. 34-35). Finally, the ILV indicated that sample dilutions were necessary to combat signal enhancement and obtain acceptable results; however, sample dilutions could not be performed for TFNG since the signal was weak (pp. 35-36, 40). The ionization source for TFNG was changed to ESI, and no signal enhancement was observed.

In the ECM, the method Limit of Quantification (LOQ) was defined as 0.1 µg/L for flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM in water (pp. 25, 31 of MRID 45854816). In the ECM, the method Limit of Detection (LOD) was defined as *ca*. 0.02 µg/L for all six analytes, based on the lowest calibration standard.

In the ILV, the method LOQ was defined as 0.1 µg/L for flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, and TFNG-AM in water (TFNA-OH was not included as an analyte; pp. 39-40 of MRID 50143203). In the ILV, the method LOD was defined as 0.05 µg/L for all five analytes, based on the lowest calibration standard. The LOQ was calculated to be 0.0966 µg/L for flonicamid (IKI-220), 0.0834 µg/L for TFNA, 0.0732 µg/L for TFNG, 0.0981 µg/L for TFNA-AM, and 0.0522 µg/L for TFNG-AM. The LOD was calculated to be 0.0322 µg/L for flonicamid (IKI-220), 0.0278 µg/L for TFNA, 0.0244 µg/L for TFNG, 0.0327 µg/L for TFNA-AM, and 0.0174 µg/L for TFNG-AM.

II. Recovery Findings

ECM 1 & 2 (MRID 45854816 & 47318701): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in two water matrices (Tables 1-12, pp. 32-46 of MRID 45854816). One ion pair transition was monitored for each analyte using LC/MS/MS in positive mode (APCI); a confirmatory method is not usually required when LC/MS and GC/MS is the primary method to generate study data. The drinking water (pH 7.42, hardness 23° DH, dissolved organic carbon 2.1 mg/L, silt content 40 mg/L) was collected from a tap at RCC; the surface water (pH 8.12, hardness 26° DH, dissolved organic carbon 2.4 mg/L, silt content 20 mg/L) was sampled from Ergolz in Lausen, Switzerland (p. 17). The First Amendment to ECM 1 MRID 45854816 was ECM 2 MRID 47318701. In the First Amendment (ECM 2 MRID 47318701), ECM 1 MRID 45854816 was modified to include the information that matrix-match calibration standards were used in the ECM 1 and that target pH of the sample prior to SPE clean-up was pH of <3 (pp. 4, 5 of MRID 47318701).

ILV (MRID 50143203): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, and TFNG-AM at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in two water matrices (Tables 1-10, pp. 45-54). TFNA-OH was not included as an analyte. One ion pair transition was monitored for each analyte using LC/MS/MS in positive mode (APCI for all analytes, except TFNG for which ESI was used); a confirmatory method is not usually required when LC/MS and GC/MS is the primary method to generate study data. The drinking water (pH not reported, hardness 122 mg/L $CaCO_3$, total dissolved solids 234 mg/L) was obtained from a faucet (municipal supply) located at GPL; surface water (pH 7.1, hardness 9 mg/L $CaCO_3$, total dissolved solids 94 mg/L) was obtained from a point on the San Joaquin River near Nees and Palm Avenues in Fresno, California (pp. 13, 22-24; Appendix C, pp. 101-113). The surface water was characterized by Agvise Laboratories, Northwood, North Dakota. Characterization data for the drinking water was provided by the City of Fresno Water Division. The ILV validated the ECM for all five analytes in the second trial for drinking water and in the third trial for surface water (p. 27). The ILV noted that failed trials were due to cross-contamination which was occurring at the rotary evaporator stations (p. 33). For the successful method validation attempts, the rotary evaporator stations were thoroughly cleaned prior to the second evaporation to prevent cross-contamination. The ILV noted that the materials used for SPE (i.e., reservoirs, valve, etc.) must not be degraded by dichloromethane or concentrated hydrochloric acid. The ILV reported that failed trials were due to the pH being >4.3 and suggested that the method be modified to emphasize that all sample pH must be suitably adjusted rather than providing a blanket adjustment step for all samples (pp. 34-35). The ILV also reported that sample dilutions were necessary to combat signal enhancement and obtain acceptable results (solvent-based calibration standards were used); however, sample dilutions could not be performed for TFNG since the signal was weak (pp. 21, 35-36, 40). The ionization source for TFNG was changed from APCI to ESI, and no signal enhancement was observed.

Table 2. Initial Validation Method Recoveries for Flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM in Water[1,2]

Analyte[3]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[4]
	Drinking Water					
Flonicamid (IKI-220)	0.1 (LOQ)	5	82.9-89.2	85.5	2.3	2.7
	1.0	5	87.0-107.7	100.8	8.2	8.1
TFNA	0.1 (LOQ)	5	**68.6**-78.2	75.2	3.9	5.2
	1.0	5	83.6-111.5	99.3	10.9	11.0
TFNG	0.1 (LOQ)	5	76.3-81.5	79.4	1.9	2.5
	1.0	5	82.3-111.0	99.9	12.2	12.2
TFNA-AM	0.1 (LOQ)	5	75.3-92.1	84.1	6.2	7.4
	1.0	5	91.6-110.0	103.8	7.3	7.0
TFNA-OH	0.1 (LOQ)	5	76.9-106.9	99.9	13.0	13.0
	1.0	5	93.8-115.5	108.7	9.0	8.3
TFNG-AM	0.1 (LOQ)	5	88.0-96.8	88.4	1.4	1.6
	1.0	5	81.5-97.7	93.5	6.8	7.3
	Surface Water					
Flonicamid (IKI-220)	0.1 (LOQ)	5	98.5-106.0	103.0	3.0	3.0
	1.0	5	97.6-104.9	102.5	3.0	2.9
TFNA	0.1 (LOQ)	5	85.7-90.7	88.5	2.2	2.4
	1.0	5	93.1-104.8	102.0	5.0	4.9
TFNG	0.1 (LOQ)	5	81.2-90.6	87.0	4.3	4.9
	1.0	5	103.0-113.0	108.9	4.3	3.9
TFNA-AM	0.1 (LOQ)	5	99.5-105.1	101.9	2.1	2.0
	1.0	5	100.4-106.7	103.7	2.5	2.4
TFNA-OH	0.1 (LOQ)	5	**68.2**-96.4	84.3	10.9	12.9
	1.0	5	100.9-110.4	105.7	4.6	4.4
TFNG-AM	0.1 (LOQ)	5	100.4-105.8	103.5	2.6	2.5
	1.0	5	96.9-105.5	102.5	3.7	3.6

Data (uncorrected recovery results; p. 24) were obtained from Tables 1-12, pp. 32-43 of MRID 45854816.

1 The drinking water (pH 7.42, hardness 23° DH, dissolved organic carbon 2.1 mg/L, silt content 40 mg/L) was collected from a tap at RCC; the surface water (pH 8.12, hardness 26° DH, dissolved organic carbon 2.4 mg/L, silt content 20 mg/L) was sampled from Ergolz in Lausen, Switzerland (p. 17).

2 Analytes were identified using one ion transition: *m/z* 229.8→203.1 for flonicamid (IKI-220), *m/z* 192.1→147.9 for TFNA, *m/z* 249.0→202.9 for TFNG, *m/z* 191.2→148.1 for TFNA-AM, *m/z* 208.1→145.9 for TFNA-OH, and *m/z* 248.0→174.0 for TFNG-AM.

3 Flonicamid = N-cyanomethyl-4-trifluoromethyl-nicotinamide; TFNA = 4-Trifluoromethylnicotinic acid; TFNG = N-(4-trifluoromethylnicotinoyl)glycine; TFNA-AM = 4-Trifluoromethylnicotinamide; TFNA-OH = 6-Hydroxy-4-trifluoromethylnicotinic acid; and TFNG-AM = N-(4-trifluoromethylnicotinoyl)glycinamide.

4 Termed “Coefficient of Variation” in study report tables (p. 26).

Table 3. Independent Validation Method Recoveries for Flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, and TFNG-AM in Water[1,2,3]

Analyte[4]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking Water					
Flonicamid (IKI-220)	0.1 (LOQ)	5	70.2-85.0	75.8	5.87	7.74
	1.0	5	71.8-85.6	79.0	6.04	7.65
TFNA	0.1 (LOQ)	5	86.0-100	94.3	5.50	5.83
	1.0	5	80.4-96.6	88.5	6.94	7.84
TFNG	0.1 (LOQ)	5	76.6-99.0	88.9	10.2	11.5
	1.0	5	72.1-90.5	84.4	7.24	8.58
TFNA-AM	0.1 (LOQ)	5	101-110	106	3.87	3.65
	1.0	5	109-117	113	3.36	2.97
TFNG-AM	0.1 (LOQ)	5	93.2-108	101	5.30	5.25
	1.0	5	91.2-107	101	5.85	5.79
	Surface Water					
Flonicamid (IKI-220)	0.1 (LOQ)	5	89.6-100	95.4	4.10	4.30
	1.0	5	91.4-102	99.0	4.33	4.37
TFNA	0.1 (LOQ)	5	70.0-105	92.4	13.7	14.8
	1.0	5	76.4-108	96.7	12.0	12.4
TFNG	0.1 (LOQ)	5	76.6-98.0	87.8	7.80	8.88
	1.0	5	82.0-94.9	90.2	5.26	5.83
TFNA-AM	0.1 (LOQ)	5	76.4-100	89.1	10.4	11.7
	1.0	5	92.8-97.6	95.5	1.80	1.88
TFNG-AM	0.1 (LOQ)	5	87.2-105	97.2	7.11	7.31
	1.0	5	95.2-104	99.3	3.20	3.22

Data (uncorrected recovery results; pp. 36-37) were obtained from Tables 3-10, pp. 45-54 of MRID 50143203.

1 The drinking water (pH not reported, hardness 122 mg/L $CaCO_3$, total dissolved solids 234 mg/L) was obtained from a faucet (municipal supply) located at GPL; surface water (pH 7.1, hardness 9 mg/L $CaCO_3$, total dissolved solids 94 mg/L) was obtained from a point on the San Joaquin River near Nees and Palm Avenues in Fresno, California (pp. 13, 22-24; Appendix C, pp. 101-113). The surface water was characterized by Agvise Laboratories, Northwood, North Dakota. Characterization data for the drinking water was provided by the City of Fresno Water Division.

2 TFNA-OH was not included as an analyte in the ILV.

3 Analytes IKI-220, TFNA, TFNA-AM, and TFNG-AM were analyzed using an APCI source, whereas TFNG was analyzed using an ESI source. Using APCI, analytes were identified using one ion transition: *m/z* 229.8→203.1 for flonicamid (IKI-220), *m/z* 191.6→148.1 for TFNA, *m/z* 190.6→148.2 for TFNA-AM, and *m/z* 247.7→174.0 for TFNG-AM. These were similar to those of the ECM. Using ESI, TFNG was identified using two ion transitions: *m/z* 249.1→203.0 for primary and *m/z* 249.1→148.0 for confirmatory; only the primary ion transition was quantified.

4 Flonicamid = N-cyanomethyl-4-trifluoromethyl-nicotinamide; TFNA = 4-Trifluoromethylnicotinic acid; TFNG = N-(4-trifluoromethylnicotinoyl)glycine; TFNA-AM = 4-Trifluoromethylnicotinamide; and TFNG-AM = N-(4-trifluoromethylnicotinoyl)glycinamide.

III. Method Characteristics

In the ECM, the method LOQ was defined as 0.1 µg/L for flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM in water (pp. 25, 31 of MRID 45854816). In the ECM, the LOQ was defined as the lowest fortification level. In the ECM, the method LOD was defined as 0.02 µg/L for all six analytes, based on the lowest calibration standard. No calculations were provided for the LOQ or LOD in the ECM.

In the ILV, the method LOQ was defined as 0.1 µg/L for flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, and TFNG-AM in water (TFNA-OH was not included as an analyte; pp. 39-40 of MRID 50143203). In the ILV, the LOQ was reported as the lowest fortification level at which acceptable recoveries were achieved. In the ILV, the method LOD was defined as 0.05 µg/L for all five analytes, based on the lowest calibration standard. The LOD and LOQ were calculated for each analyte using the following equations:

$LOD = (t_{0.99} \times SD)$

$LOQ = 3 \times LOD$

Where, $t_{0.99}$ is the t value for n-1 replicates at the 99% confidence level and SD is the standard deviation of the analyte recovery measurements at the target LOQ using combined data (both water matrices; n =10).

The LOQ was calculated to be 0.0966 µg/L for flonicamid (IKI-220), 0.0834 µg/L for TFNA, 0.0732 µg/L for TFNG, 0.0981 µg/L for TFNA-AM, and 0.0522 µg/L for TFNG-AM. The LOD was calculated to be 0.0322 µg/L for flonicamid (IKI-220), 0.0278 µg/L for TFNA, 0.0244 µg/L for TFNG, 0.0327 µg/L for TFNA-AM, and 0.0174 µg/L for TFNG-AM. Calculated LOQs and LODs supported the method LOQ and LOD.

Table 4. Method Characteristics for Flonicamid (IKI-220), TFNA, TFNG, TFNA-AM, TFNA-OH, and TFNG-AM in Water[1,2]

Parameter			Flonicamid (IKI-220)	TFNA	TFNG	TFNA-AM	TFNG-AM	TFNA-OH
Limit of Quantitation (LOQ)	ECM		0.1 µg/L					
	ILV		0.1 µg/L					Not included
			0.0966 µg/L	0.0834 µg/L	0.0732 µg/L	0.0981 µg/L	0.0522 µg/L	
Limit of Detection (LOD)[1]	ECM		0.02 µg/L					
	ILV		0.05 µg/L					Not included
			0.0322 µg/L	0.0278 µg/L	0.0244 µg/L	0.0327 µg/L	0.0174 µg/L	
Linearity (calibration curve r^2 and concentration range)	ECM[3]		r^2 = 0.997-0.999	r^2 = 0.996	r^2 = **0.994**-0.996	r^2 = 0.997-0.999	r^2 = 0.995-0.997	r^2 = **0.994**-0.999
			0.01-1.0 µg/mL					
	ILV	Surface	r^2 = 0.9984	r^2 = 0.9984	r^2 = 0.9994	r^2 = 0.9984	r^2 = 0.9992	Not included
		Drinking	r^2 = 0.9986	r^2 = 0.9992	r^2 = 0.9988	r^2 = 0.9984		
			0.25-010.0 µg/L					
Repeatable	ECM[4,5]		Yes, at LOQ and 10×LOQ. (two characterized water matrices used)					
	ILV[6,7]		Yes, at LOQ and 10×LOQ. (two characterized water matrices used)					**No**, no samples prepared.
Reproducible			Yes, at LOQ and 10×LOQ.					**Could not be determined**; only one set of performance data was submitted.
Specific	ECM		Yes, matrix interferences were <2% of the LOQ (based on peak area).	Yes, matrix interferences were *ca.* 5% of the LOQ (based on peak area).	Yes, matrix interferences were <2% of the LOQ (based on peak area).	Yes, matrix interferences were *ca.* 2% of the LOQ (based on peak area). Minor baseline noise was observed at LOQ.	Yes, matrix interferences were *ca.* 2% of the LOQ (based on peak area).	Yes, no matrix interferences were observed, but minor baseline noise was observed at LOQ.

Parameter		Flonicamid (IKI-220)	TFNA	TFNG	TFNA-AM	TFNG-AM	TFNA-OH
	ILV	Yes, no matrix interferences were observed, but minor baseline noise was observed at LOQ.	Yes, no matrix interferences were observed, but LOQ peak height was only 2-5xs the height of minor contaminants' peak height.	Yes, no matrix interferences were observed, but minor baseline noise was observed at LOQ.	Yes, no matrix interferences were observed, but LOQ peak height was only 2-5xs the height of minor contaminants' peak height.	Yes, no matrix interferences were observed, but minor baseline noise was observed at LOQ.	**Not included**

Data were obtained from pp. 25, 31 (LOQ/LOD); p. 29 (calibration data); Tables 1-12, pp. 32-43 (recovery results); Tables 13-18, pp. 44-49 (calibration curve); Figures 1-8, pp. 50-57 (chromatograms) of MRID 45854816; pp. 39-40 (LOQ/LOD); Tables 1-10, pp. 45-54 (recovery results); Appendix D, pp. 130-134 and 140-144 (calibration data); Appendix E, pp. 146-226 (chromatograms) of MRID 50143203; DER Attachment 2.

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

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

1 Flonicamid = N-cyanomethyl-4-trifluoromethyl-nicotinamide; TFNA = 4-Trifluoromethylnicotinic acid; TFNG = N-(4-trifluoromethylnicotinoyl)glycine; TFNA-AM = 4-Trifluoromethylnicotinamide; and TFNG-AM = N-(4-trifluoromethylnicotinoyl)glycinamide.

2 In the ECM 1, one ion pair transition was monitored for each analyte using LC/MS/MS in positive mode (APCI); in the ILV, one ion pair transition was monitored for each analyte using LC/MS/MS in positive mode (APCI for all analytes, except TFNG for which ESI was used).

3 Ranges were reported for the two correlation coefficients provided for each analyte. The two values corresponded to different calibration curve dates.

4 In the ECM 1, the drinking water (pH 7.42, hardness 23° DH, dissolved organic carbon 2.1 mg/L, silt content 40 mg/L) was collected from a tap at RCC; the surface water (pH 8.12, hardness 26° DH, dissolved organic carbon 2.4 mg/L, silt content 20 mg/L) was sampled from Ergolz in Lausen, Switzerland (p. 17 of MRID 45854816).

5 ECM 2 MRID 47318701 contained supplemental information for ECM 1 MRID 45854816. The supplemental information contained method procedure details and no additional data.

6 In the ILV, the drinking water (pH not reported, hardness 122 mg/L CaCO3, total dissolved solids 234 mg/L) was obtained from a faucet (municipal supply) located at GPL; surface water (pH 7.1, hardness 9 mg/L CaCO3, total dissolved solids 94 mg/L) was obtained from a point on the San Joaquin River near Nees and Palm Avenues in Fresno, California (pp. 13, 22-24; Appendix C, pp. 101-113 of MRID 50143203). The surface water was characterized by Agvise Laboratories, Northwood, North Dakota. Characterization data for the drinking water was provided by the City of Fresno Water Division.

7 The ILV validated the ECM for all five analytes (flonicamid IKI-220, TFNA, TFNG, TFNA-AM, and TFNG-AM) in the second trial for drinking water and in the third trial for surface water (p. 27 of MRID 50143203). The ILV noted that failed trials were due to cross-contamination at the rotary evaporator stations and to the pH being >4.3 and suggested that the method be modified to emphasize thorough cleaning of all equipment and that all sample pH must be suitably adjusted rather than providing a blanket adjustment step for all samples (pp. 33-35). The ILV also reported that sample dilutions were necessary to combat signal enhancement and obtain acceptable results (solvent-based calibration standards were used); however, sample dilutions could not be performed for TFNG since the signal was weak (pp. 21, 35-36, 40). The ionization source for TFNG was changed from APCI to ESI, and no signal enhancement was observed.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV removed flonicamid metabolite TFNA-OH from the protocol of the independent validation of the method because the study author noted that TFNA-OH is only an animal metabolite (p. 42 of MRID 50143203). However, this is a major degradate in an aerobic soil metabolism study (MRID 45656804) and was also observed in an aerobic aquatic metabolism study MRID 45854822). Due to the exclusion of TFNA-OH in the ILV, the reproducibility of the method for TFNA-OH could not be determined since only one set of performance data was submitted, that of the ECM. OCSPP guidelines state that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV.

2. The ILV reported that failed trials were due to the pH being >4.3 and suggested that the method be modified to emphasize that all sample pH must be suitably adjusted rather than providing a blanket adjustment step for all samples (pp. 34-35 of MRID 50143203). The ILV also reported that sample dilutions were necessary to combat signal enhancement and obtain acceptable results (solvent-based calibration standards were used); however, sample dilutions could not be performed for TFNG since the signal was weak (pp. 21 ,35-36, 40). The ionization source for TFNG was changed from APCI to ESI, and no signal enhancement was observed.

3. The communications between Jason McDonald, the Sponsor Representative, Elisabeth Schoenau, the Study Director, and Robert Testman, the Testing Facility Management involved trial results (including chromatograms) and reasons for the failed trials (pp. 40-42 of MRID 50143203). Detailed communication records were reportedly detailed in the raw data, but not provided. All ILV issues were resolved as a result of this communication.

4. ECM linearity was not satisfactory for one of the calibration curves of TFNG ($r^2 = 0.9943$; p. 49 of MRID 45854816) and TFNA-OH ($r^2 = 0.994$; p. 32 of MRID 45854816). Linearity is satisfactory when $r^2 \geq 0.995$.

5. The specificity of the method for TFNA and TFNA-AM was not well-supported by ILV representative chromatograms since LOQ peak height was only 2 to 5 times the height of minor contaminants' peak height (Appendix E, pp. 146-226 of MRID 50143203).

6. 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 (pp. 25, 31 of MRID 45854816; pp. 39-40 of MRID 50143203). In the ECM, the LOQ was defined as the lowest fortification level. In the ECM, the method LOD was based on the lowest calibration standard. No calculations were provided for the LOQ or LOD in the ECM. In the ILV, the LOQ was reported as the lowest fortification level at which acceptable recoveries were achieved. In the ILV, the method LOD was based on the lowest calibration standard. The LOD and LOQ were calculated for each analyte using the following equations: LOD = ($t_{0.99}$ x SD) and LOQ = 3 x LOD, where $t_{0.99}$ is the t value for n-1 replicates at the 99% confidence level and SD is the standard deviation of the analyte recovery measurements at the target LOQ using combined data (both water matrices; n =10). Calculated LOQs and LODs supported the method LOQ and LOD.

7. The matrix effects were not measured in the ILV and ECM. In the ILV signal enhancement

was observed for IKI-220, TFNG, and TFNG-AM (p.35 MRID 50143203). An experiment was conducted to confirm that signal enhancement was occuring for these three analytes. A 10x LOQ surface water sample was diluted and injected against a two-point calibration curve which showed that signal enhancement was improved by dilution. All surface water extracts from the ILV trial 2 were diluted and reanalyzed. The 10-fold dilution suitably improved the signal for IKI-220 and TFNG-AM, but not for TFNG. Average recoveries for TFNG were 385% and the signal was not strong enough for further dilution. The signal for TFNG was improved by changing the ionization source from APCI to ESI.

8. The ECM noted that the analytes appeared stable in the sample extracts for up to 3 days in a refrigerator at *ca*. 4°C based on the acceptable recovery results obtained (p. 31 of MRID 45854816). The "Herndon-39-151214" sample was used for the trial 1 and 2 surface water method validation attempt. This site where the first surface water control matrix sample was obtained did not have water at the time of the second sampling. As a result, the second surface water control matrix sample was obtained from a point on the San Joaquin River near Nees and Palm Avenues in Fresno, California.

9. In the ECM, first surface water control matrix sample was obtained from the Fresno Irrigation District Canal "Herndon No. 39", near the Gates Avenue Bridge (p. 23 of MRID 50143203).

10. The time required to complete the method was not reported in the ECM. Based on the ILV report, it takes 6 to10 hours for one person to prepare an analysis set from sample preparation to instrumental analysis and an additional two hours for data calculation and tabulation. The ILV notes that reduction of the sample size from 1 L to 100 mL is suggested to shorten the time needed to prepare a sample set from 10 hours to 6 hours (p. 34 of MRID 50143203).

V. References

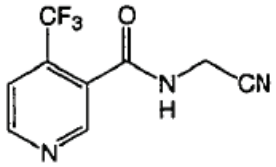
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

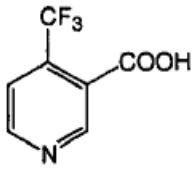
Flonicamid (IKI-220; F-1785)

IUPAC Name: N-cyanomethyl-4-trifluromethyl-nicotinamide
CAS Name: N-(cyanomethyl)-4-(trifluromethyl)-3-pyridine-carboxamide
CAS Number: 158062-67-0
SMILES String: Not found



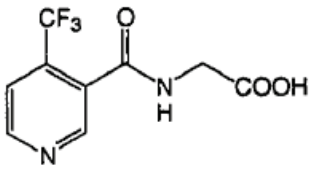
TFNA

IUPAC Name: 4-Trifluromethylnicotinic acid
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



TFNG

IUPAC Name: N-(4-trifluromethylnicotinoyl)glycine
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



TFNA-AM

IUPAC Name: 4-Trifluromethylnicotinamide
CAS Name: Not reported
CAS Number: Not reported
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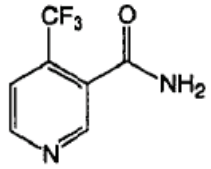
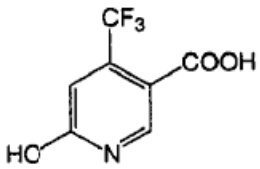


TFNA-OH

IUPAC Name: 6-Hydroxy-4-trifluromethylnicotinic acid
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



TFNG-AM

IUPAC Name: N-(4-trifluromethylnicotinoyl)glycinamide
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found