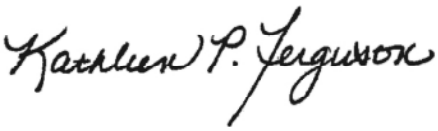
ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Test Material:	Fluazinam
MRID:	48635802
Title:	Robaugh, D.A. 2011. Independent laboratory validation of enforcement method for the analysis of fluazinam and five metabolites in water.
MRID:	48635802 – Appendix A
Title:	Schoenau, E.A. 2011. Analytical procedure for the determination of fluazinam and five metabolites (AMPA, DAPA, CAPA, DCPA, HYPA) in water. GLP-MTH-077.
EPA PC Code:	129098
OCSPP Guideline:	850.6100

For Cambridge Environmental

Primary Reviewer: Lynne Binari **Signature:** 

Date: 5/14/12

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 5/14/12

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 5/14/12

Data Requirement: EPA Guideline: 850.6100
OECD Data Point: IIA 4.5

Test material:
Common name: Fluazinam
Chemical name: 3-Chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine.
IUPAC: 3-Chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-p-toluidine (Appendix A, p. 88).

Final Reviewer: José L. Meléndez **Signature:** José Luis Meléndez

Environmental Risk Branch V **Date:** 04/09/2013

ANALYTICAL METHOD: EPA MRID No. 48635802 – Appendix A. Schoenau, E.A. 2011. Analytical procedure for the determination of fluazinam and five metabolites (AMPA, DAPA, CAPA, DCPA, HYPA) in water. GPL-MTH-077. Report prepared by Golden Pacific Laboratories, LLC, Fresno, California, sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan, and submitted by ISK Biosciences Corporation, Concord, Ohio; 15 pages (p. 1A; Appendix A, pp. 87-101). Final report issued May 24, 2011 (Appendix A, p. 87).

INDEPENDENT LABORATORY VALIDATION: EPA MRID No. 48635802. Robaugh, D.A. 2011. Independent laboratory validation of enforcement method for the analysis of fluazinam and five metabolites in water. Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado, sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan, and submitted by ISK Biosciences Corporation, Concord, Ohio; 120 pages. Final report issued September 30, 2011.

SYNOPSIS

This analytical method, "Analytical procedure for the determination of fluazinam and five metabolites (AMPA, DAPA, CAPA, DCPA, HYPA) in water", is designed for the quantitative determination of fluazinam and its transformation products AMPA, DAPA, CAPA, DCPA, HYPA in water using LC/MS/MS (see **Table 1**). The method appears to be quantitative for the above mentioned compounds at the stated LOQ of 0.10 µg/L for each analyte. The LOQs are less than the lowest toxicological level of ecological concern in water. However, issues were noted as indicated below under "Method Acceptability". The data on the ECM were incomplete. The registrant should address these issues in order to upgrade the study.

Table 1. Analytical Method Summary

Analyte	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluazinam, AMPA, DAPA, CAPA, DCPA, HYPA	48635802, Appendix A	48635802		Water	05/24/11	ISK Biosciences Corp.	LC/MS/ MS	0.10 µg/L[1]

1. The registrant must provide additional data regarding the environmental chemistry method. Available data are incomplete.

EXECUTIVE SUMMARY

This method is designed for the quantitative determination of residues of fluazinam and its five transformation products AMPA, DAPA, CAPA, DCPA and HYPA in water. The method was validated by Golden Pacific Laboratories, LLC, for Ishihara Sangyo Kaisha, Ltd.; no regulatory guidelines were cited in the ECM (Appendix A, pp. 87-101). An independent laboratory validation (ILV), performed by Pyxant Labs Inc., was submitted with the method. The Agency finds that this method is **supplemental** for fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA. The registrant must provide the analytical results of the environmental chemistry method and address certain issues found, as described below under "Method Acceptability".

Method Summary: Water is combined with 0.2% formic acid in acetonitrile at a ratio of 9:1 (v:v), then analyzed directly for fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA using LC/MS/MS (Appendix A, pp. 90, 92). The ECM defined a limit of quantitation (LOQ) of 0.10 µg/L for the six analytes in water, with a limit of detection (LOD) of 0.0556 µg/L, which were supported by the ILV (Appendix A, p. 97).

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

For the ECM, performance data were incomplete with only results from one water sample fortified at the LOQ with all six compounds reported (Appendix A, pp. 99-101). Chromatograms of standards and method and matrix blank samples were not provided. Calibration curves and linear regression analyses were not provided. Results from the confirmatory method were not reported. The source and characterization of the water matrix were not reported.

For the ILV, acceptance criteria were met (matrix spike recoveries ranging between 70% to 120% and relative standard deviations of ≤20%) at the LOQ and 10 x LOQ for all analytes, except for two of the five fortifications for HYPA at the LOQ (Tables 1-6, pp. 23-26). Quantitative results from the confirmatory method were not reported and data on the representative chromatograms were illegible (Figures 49-54, pp. 81-86). The tap water matrix was not further characterized.

The ILV found that no substitutions should be made for glass equipment due to adsorption of all six analytes to plastics.

COMPLIANCE

No regulatory guidelines were cited in the ECM.

A. BACKGROUND INFORMATION

TABLE A.1. Test Compound Nomenclature

Parameter	Value
Common name	**Fluazinam**
Company experimental name	IKF-1216 PAI, IKF-1216, B1216, PP192 (p. 1A; Appendix B, p. 102).
IUPAC name	3-Chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-p-toluidine.
CAS Name	3-Chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine.

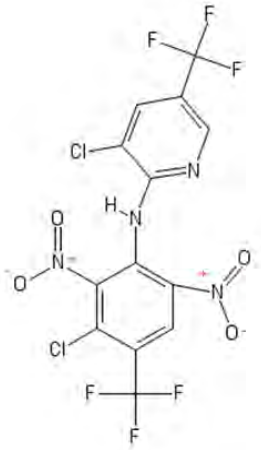
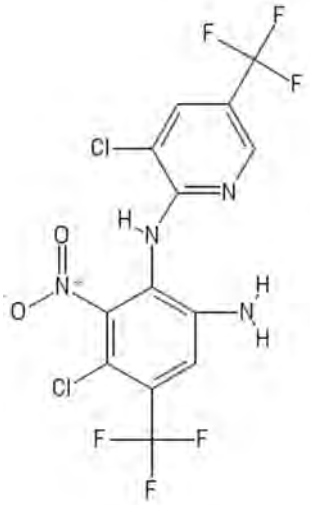
TABLE A.1. Test Compound Nomenclature	
Parameter	**Value**
CAS #	79622-59-6
Structure	
Common name	**AMPA**
Company experimental name	IKF-1216 Metabolite-AMPA (Appendix B, p. 103).
IUPAC name	2-(6-Amino-3-chloro-α,α,α-trifluoro-2-nitro-p-toluidino)-3-chloro-5-(trifluoro-methyl)pyridine.
CAS Name	2-(6-Amino-3-chloro-α,α,α-trifluoro-2-nitro-ρ-toluidino)-3-chloro-5-trifluoro-methylpyridine.
CAS #	Not reported.
Structure	
Common name	**DAPA**
Company experimental name	IKF-1216 Metabolite-DAPA (Appendix B, p. 104).

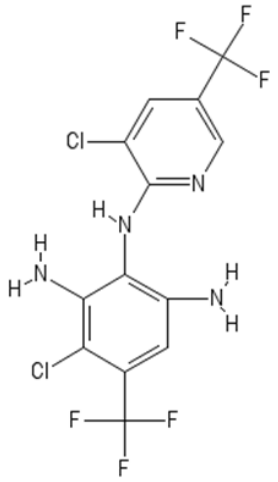
TABLE A.1. Test Compound Nomenclature	
Parameter	**Value**
IUPAC name	3-Chloro-2-(2,6-diamino-3-chloro-α,α,α-trifluoro-p-toluidino)-5-(trifluoromethyl)-pyridine.
CAS Name	3-Chloro-2-(2,6-diamino-3-chloro-α,α,α-trifluoro-ρ-toluidino)-5-trifluoromethyl-pyridine.
CAS #	Not reported.
Structure	
Common name	**CAPA**
Company experimental name	None reported (Appendix B, p. 105).
IUPAC name	5-Chloro-6-(3-chloro-α,α,α-trifluoro-2,6-dinitro-p-toluidino)-nicotinic acid.
CAS Name	5-Chloro-6-[[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid.
CAS #	Not reported.

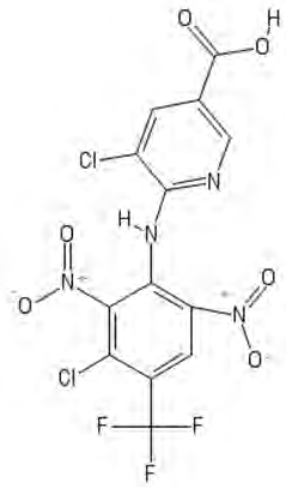
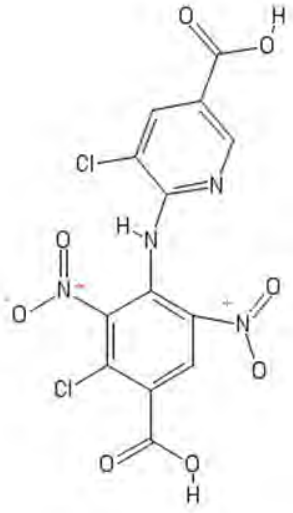
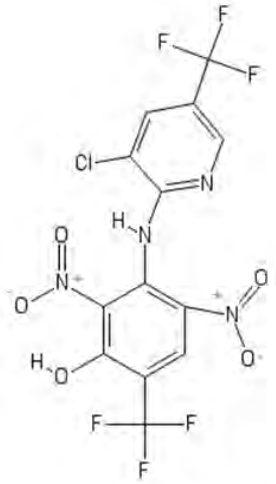
TABLE A.1. Test Compound Nomenclature	
Parameter	**Value**
Structure	
Common name	**DCPA**
Company experimental name	None reported (Appendix B, p. 106).
IUPAC name	6-(4-Carboxy-3-chloro-2,6-dinitroanilino)-5-chloronicotinic acid.
CAS Name	6-[(4-Carboxy-3-chloro-2,6-dinitrophenyl)-amino]-5-chloro-3-pyridinecarboxylic acid.
CAS #	Not reported.
Structure	
Common name	**HYPA**
Company experimental name	None reported (Appendix B, p. 107).
IUPAC name	5-((3-Chloro-5-(trifluoromethyl)-2-pyridyl)amino)-α,α,α-trifluoro-4,6-dinitro-o-cresol.

TABLE A.1. Test Compound Nomenclature	
Parameter	**Value**
CAS Name	3-[[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]amino]-2,4-dinitro-6-(trifluoro-methyl)phenol.
CAS #	79614-99-6
Structure	

Information obtained from p. 1A; Appendix B, pp. 102-107 of the study report. Except for DCPA, CAS names obtained from Fluazinam structures[1].doc, as well as HYPA CAS No.

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound	
Parameter	**Value**
Melting point/range (ºC)	Not reported.
pH	Not reported.
Density (g/cm^3)	Not reported.
Water solubility at 20ºC (mg/L)	Not reported.
Solvent solubility at 20 ºC (mg/L)	Not reported.
Vapor pressure at __ºC (torr)	Not reported.
Dissociation constant (pK_a)	Not reported.
Octanol/water partition coefficient	Not reported.
UV/visible absorption spectrum (nm)	Not reported.

B. MATERIALS AND METHODS

B.1. Principle of Method

The water sample is combined with 0.2% formic acid in acetonitrile at a ratio of 9:1 (v:v), then analyzed directly for fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA by LC/MS/MS-ESI$^+$ using a Phenomenex Synergi Polar-RP column (Appendix A, pp. 92-

93). For each compound, two ion transitions are monitored for quantitation and confirmation (Appendix A, pp. 94-95).

TABLE B.1. Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied

Parameter	Value
Method ID	Analytical procedure for the determination of fluazinam and five metabolites (AMPA, DAPA, CAPA, DCPA, HYPA) in water. GPL-MTH-077 (Appendix A, p. 87).
Analyte(s)	Fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA.
Extraction solvent/technique	Water (9 mL) is transferred to a glass tube, combined with 0.2% formic acid in acetonitrile (1 mL) and shaken for 30 seconds (Appendix A, p. 92).
Cleanup strategies	As needed, filter sample through glass fiber filter (1-µm, 25-mm) using a glass syringe to remove particulates (Appendix A, pp. 90, 92).
Instrument/Detector	Shimadzu HPLC system with Phenomenex Synergi Polar-RP column (2 x 50 mm, 4-µm, 80 Å) and Applied Biosystems (AB) Sciex API5000 LC/MS/MS equipped with Turbo Spray electrospray ionization in positive ion mode (ESI^+) and multiple reaction monitoring (MRM; Appendix A, pp. 90, 92-93).

Information obtained from Appendix A, pp. 87, 90, 92-93 of the study report.

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1. Recovery Results from Method Validation for the Determination of Fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA in Water (n = 1)[1]

Analyte	Spiking Level (µg a.i./L)	Recoveries Obtained (%)	Relative Standard Deviation
Fluazinam	0.10 (LOQ)	102	--
	1.0	--	--
AMPA	0.10 (LOQ)	96.9	--
	1.0	--	--
DAPA	0.10 (LOQ)	102	--
	1.0	--	--
CAPA	0.10 (LOQ)	95.2	--
	1.0	--	--
DCPA	0.10 (LOQ)	72.8	--
	1.0	--	--
HYPA	0.10 (LOQ)	85.5	--
	1.0	--	--

Results from Appendix A, pp. 99-101 of the study report.
-- = Not reported.
1 Only results from one water sample fortified at a nominal 0.1 µg a.i./L were reported (Appendix A, pp. 99-101). Results could not be verified by reviewer because supporting data were not provided.

Results from the confirmatory method were not reported.

C.1.1. Method Characteristics

TABLE C.2. Method Characteristics	
Parameter	**Value**
Analyte(s)	Fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA.
Limit of Quantitation (LOQ)	0.10 µg a.i./L (Appendix A, p. 97).
Limit of Detection (LOD)	0.0556 µg a.i./L (Appendix A, p. 97).
Accuracy/Precision at LOQ	ECM: Acceptance criteria (EFED-ECM 2, Version 1, December 2010, p. 5; OCSPP 850.6100) were met at the LOQ with matrix spike recoveries ranging between 70% to 120%; however, relative standard deviations were not reported and could not be determined by the reviewer due to insufficient reported results (Appendix A, pp. 99-101).
Reliability of the Method/[ILV]	ILV: For fluazinam, AMPA, DAPA, DAPA and DCPA, acceptance criteria were met at the LOQ with matrix spike recoveries ranging between 70% to 120% and relative standard deviations of ≤20% (Tables 1-5, pp. 23-25). For HYPA, two of the five fortifications had recoveries >120% (LOQ-1 121%, LOQ-5 123%; Table 6, p. 26). The method was validated after two trials. In the first trial, fluazinam and its products were found to adsorb to polypropylene (p. 20).
Linearity	ECM: not reported. ILV: Linear regression: r = 0.9957-0.9994 (p. 17; Figures 1-6, pp. 33-38).
Specificity	ECM: could not be determined because quantitative results and chromatograms for standards and method and matrix blank samples were not provided. ILV: Comparison of chromatograms produced for standards and control and fortified samples demonstrates that the method, based on LC/MS/MS, is highly specific for the analysis of fluazinam and its products AMPA, DAPA, CAPA, DCPA and HYPA (Figures 7-48, pp. 39-80). Method and matrix blank controls showed no significant interferences (<0.01 µg/L) at the retention times of the six analytes (Tables 7-12, pp. 27-32; Figures 13-24, pp. 45-56; DER Attachment 2).

Information obtained from pp. 17, 20; Tables 1-12, pp. 23-32; Figures 1-48, pp. 33-80; Appendix A, pp. 97, 99-101 of the study report and DER Attachment 2.

C.2. Independent Laboratory Validation (ILV)

The ILV was conducted in compliance with USEPA GLP Standards 40 CFR, Part 160, OPPTS 850.7100 and OPPTS 835.6200 guidelines, and PR Notice 96-1 (pp. 3, 12, 21-22). [Reviewed under OCSPP 850.6100.]

TABLE C.3. Recovery Results of the Method Obtained by an Independent Laboratory Validation for the Determination of Residues in Tap Water (n = 5)

Analyte	Spiking Level (µg a.i./L)	Mean Recoveries Obtained (%)	Relative Standard Deviation
Fluazinam	0.10 (LOQ)	86	0.94
	1.0	94	4.5
AMPA	0.10 (LOQ)	111	1.2
	1.0	101	2.3
DAPA	0.10 (LOQ)	99	2.4
	1.0	103	1.8
CAPA	0.10 (LOQ)	110	2.9
	1.0	102	1.6
DCPA	0.10 (LOQ)	109	7.0
	1.0	106	6.1
HYPA	0.10 (LOQ)	116	5.7
	1.0	112	3.5

Results obtained from Tables 1-6, pp. 23-26; reported results verified by reviewer (DER Attachment 2).

Quantitative results from the confirmatory method were not reported and data on representative chromatograms were illegible (Figures 49-54, pp. 81-86).

D. CONCLUSION

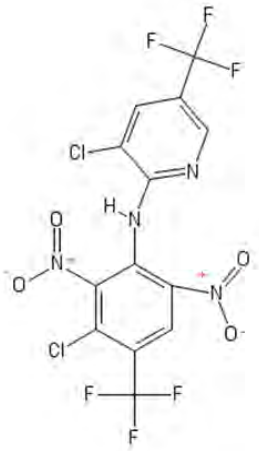
This method is designed for the quantitative determination of residues of fluazinam and its products AMPA, DAPA, CAPA, DCPA and HYPA in water. The Agency finds that this method is **supplemental** for fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA in water. For HYPA, two of the five fortifications at the LOQ were not within acceptance criteria (*i.e.*, <70% or >120% recovery; EFED-ECM 2, Version 1, December 2010, p. 5; OCSPP 850.6100).

[Refer to the attached review checklist.]

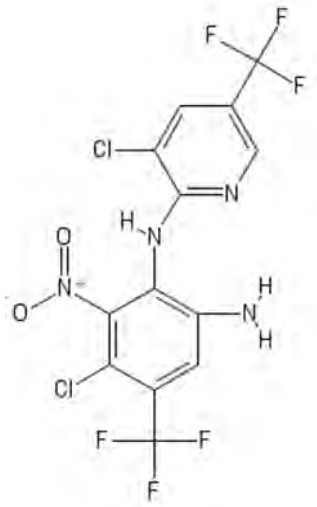
ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

ENVIRONMENTAL CHEMISTRY METHOD (ECM) STANDARD EVALUATION PROCEDURE (SEP) CHECKLIST: BACKGROUND AND INITIAL REVIEW INFORMATION

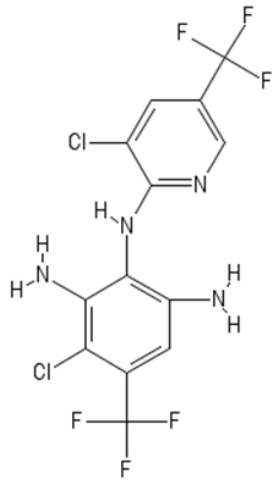
I. Background Information

A.	**Title of Method**	Analytical procedure for the determination of fluazinam and five metabolites (AMPA, DAPA, CAPA, DCPA, HYPA) in water. GPL-MTH-077 (Appendix A, p. 87).	
B.	**ECM No.** [ECB use]		
C.	**MRID No.**	48635802	
D.	**Matrix**	Water	
E.	**Analyte(s) detected**	**Compound:**	
		Common name:	Fluazinam
		IUPAC name:	3-Chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-p-toluidine (Appendix B, p. 102).
		CAS name:	3-Chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine.
		CAS No:	79622-59-6
		Synonyms:	IKF-1216 PAI, IKF-1216, B1216, PP192 (p. 1A; Appendix B, p. 102).
			

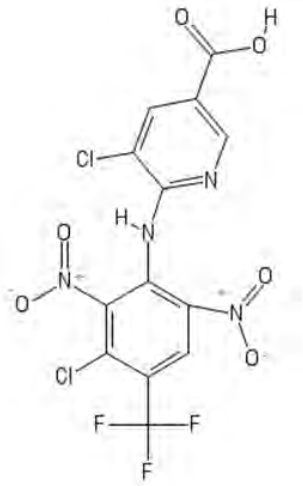
ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

Compound:	
Common name:	AMPA
IUPAC name:	2-(6-Amino-3-chloro-α,α,α-trifluoro-2-nitro-p-toluidino)-3-chloro-5-(trifluoro-methyl)pyridine (Appendix B, p. 103).
CAS name:	2-(6-Amino-3-chloro-α,α,α-trifluoro-2-nitro-ρ-toluidino)-3-chloro-5-trifluoro-methylpyridine.
CAS No:	Not reported.
Synonyms:	IKF-1216 Metabolite-AMPA
	

Compound:	
Common name:	DAPA
IUPAC name:	3-Chloro-2-(2,6-diamino-3-chloro-α,α,α-trifluoro-p-toluidino)-5-(trifluoromethyl)-pyridine (Appendix B, p. 104).
CAS name:	3-Chloro-2-(2,6-diamino-3-chloro-α,α,α-trifluoro-ρ-toluidino)-5-trifluoromethyl-pyridine.
CAS No:	Not reported.
Synonyms:	IKF-1216 Metabolite-DAPA

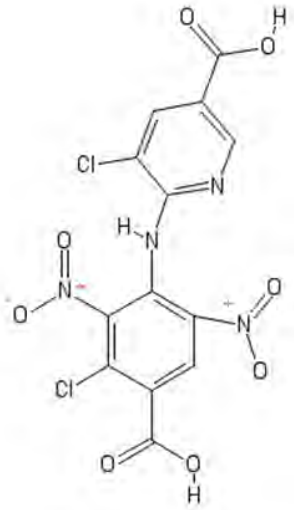


Compound:	
Common name:	CAPA
IUPAC name:	5-Chloro-6-(3-chloro-α,α,α-trifluoro-2,6-dinitro-p-toluidino)-nicotinic acid (Appendix B, p. 105).
CAS name:	5-Chloro-6-[[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid.
CAS No:	Not reported.
Synonyms:	None reported.



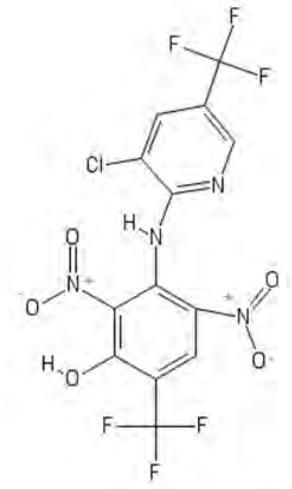
Compound:

Common name:	DCPA
IUPAC name:	6-(4-Carboxy-3-chloro-2,6-dinitroanilino)-5-chloronicotinic acid (Appendix B, p. 106).
CAS name:	6-[(4-Carboxy-3-chloro-2,6-dinitrophenyl)-amino]-5-chloro-3-pyridinecarboxylic acid.
CAS No:	Not reported.
Synonyms:	None reported.



Compound:	
Common name:	HYPA
IUPAC name:	5-((3-Chloro-5-(trifluoromethyl)-2-pyridyl)amino)-α,α,α-trifluoro-4,6-dinitro-o-cresol (Appendix B, p. 107).
CAS name:	3-[[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]amino]-2,4-dinitro-6-(trifluoro-methyl)phenol.
CAS No:	79614-99-6
Synonyms:	None reported.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

Information obtained from p. 1A; Appendix A, p. 87; Appendix B, pp. 102-107 of the study report. Except for DCPA, CAS names obtained from Fluazinam structures[1].doc, as well as HYPA CAS No.

II. Information about the Laboratory

A.	**Name**	Golden Pacific Laboratories, LLC (Appendix A, p. 87).
B.	**Address**	4720 West Jennifer Ave., Suite 105, Fresno, California 93722.
C.	**Telephone No.**	559-275-9091
D.	**Name of the Study Director**	Not reported.
E.	**Name of the Lead Chemist**	Elisabeth A. Schoenau (Appendix A, p. 87).
F.	**Laboratory Validation:**	Not provided.

Information obtained from Appendix A, p. 87 of the study report; address and telephone number from GPL website.

III. Method Summary Information for Analyte(s): **Fluazinam and its products AMPA, DAPA, CAPA, DCPA and HYPA.**

A.	**Statement of Data Confidentiality**	Yes (p. 2).
1.	**Is the Method Classified or Confidential?**	No.
2.	**Submitted Prior to 2008 with a Non-Standard Claim of Confidentiality?**	No.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

B.	**Sample Preparation**	Samples should not be collected or analyzed in plastic bottles as fluazinam and AMPA may absorb to the plastic (Appendix A, p. 92). Water (100 mL) was fortified with a mixed standard solution of fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA, in acetonitrile, at 0.10 and 1.0 µg a.i./L (Appendix A, pp. 91-92). Application solution volumes were not reported.
C.	**Sample Extraction**	Water (9 mL) transferred to a glass tube, combined with 0.2% formic acid in acetonitrile (1 mL) and shaken for 30 seconds (Appendix A, p. 92).
D.	**Sample Cleanup**	As needed, filter sample through glass fiber filter (1-µm, 25-mm) using a glass syringe to remove particulates (Appendix A, pp. 90, 92).
E.	**Sample Derivatization (if applicable)**	None reported.
F.	**Sample Analysis**	LC/MS/MS (Appendix A, p. 92).
1.	**Instrumentation**	Shimadzu HPLC system and Applied Biosystems (AB) Sciex API5000 LC/MS/MS equipped with Turbo Spray electrospray ionization in positive ion mode (ESI^+; Appendix A, pp. 90, 92-93).
2.	**Primary Column**	Phenomenex Synergi Polar-RP column (2 x 50 mm, 4 µm, 80 Å; Appendix A, p. 93).
3.	**Confirmatory Column (if any)**	None reported.
4.	**Detector**	Multiple Reaction Monitoring (MRM; Appendix A, p. 93).
5.	**Other Confirmatory Techniques (if any)**	For each compound, two ion transitions were monitored for quantitation and confirmation (Appendix A, pp. 94-95).
6.	**Other Relevant Information**	See nested table below.
G.	**Detection and Quantitation Limits**	

Compound	Ions monitored (m/z)		Retention time (min.)
	Quantitation	Confirmation	
Fluazinam	465.0 > 373.0	465.0 > 338.0	*ca.* 4.3
AMPA	435.0 > 373.0	435.0 > 354.0	*ca.* 4.0
DAPA	405.0 > 333.0	405.0 > 353.0	*ca.* 3.8
CAPA	441.0 > 349.0	441.0 > 303.0	*ca.* 3.6
DCPA	417.0 > 325.1	417.0 > 279.0	*ca.* 2.4
HYPA	447.1 > 382.9	447.1 > 355.1	*ca.* 3.4

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

1.	**Limit of Quantitation (LOQ)**			
	Claimed in Method	0.1 µg/L (Appendix A, p. 97).	**Estimated**	No justification for selected LOQ was provided.
2.	**Limit of Detection (LOD)**			
	Claimed in Method	0.0556 µg/L (Appendix A, p. 97).	**Estimated**	Estimated from lowest calibration standard (0.05 µg/L; Appendix A, p. 91).

H.	**Recovery (Accuracy)/Precision Data; expressed as percentage of applied (n = 1)[1]**							
	Spiking Level (µg a.i./L)	**Parameter**	**Fluazinam**	**AMPA**	**DAPA**	**CAPA**	**DCPA**	**HYPA**
	0.1 (LOQ)	**Range**	102	96.9	102	95.2	72.8	85.5
		Mean	--	--	--	--	--	--
		SD	--	--	--	--	--	--
		RSD	--	--	--	--	--	--
	1.0	**Range**	--	--	--	--	--	--
		Mean	--	--	--	--	--	--
		SD	--	--	--	--	--	--
		RSD	--	--	--	--	--	--

Information obtained from p. 2; Appendix A, pp. 90-95, 97, 99-101 of the study report.
-- = Not reported.
1 Only results from one water sample fortified at a nominal 0.1 µg a.i./L were reported (Appendix A, pp. 99-101). Results could not be verified by reviewer because supporting data were not provided.

IV. Detailed Information about the Method

		YES	**NO**	**REVIEW FURTHER**
A.	**Does the method require spiking with the analytes(s) of interest?**	x		Appendix A, p. 92.
B.	**If the method requires explosive or carcinogenic reagents, are proper precautions explained?**			Not applicable.
C.	**Is the following information supplied?**			

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
1.	**Detailed stepwise description of:**			
a.	**The sample preparation procedure?**	x		Appendix A, p. 92.
b.	**The sample spiking procedure?**	x		Application solution volumes not reported (Appendix A, p. 92).
c.	**The extraction procedure?**	x		Appendix A, p. 92.
d.	**The derivatization procedure?**			Not applicable.
e.	**The clean-up procedure?**	x		Appendix A, p. 92.
f.	**The analysis procedure?**	x		Appendix A, pp. 92-95.
2.	**Procedures for:**			
a.	**Preparation of standards?**	x		Appendix A, p. 91.
b.	**Calibration of instrument?**	x		Appendix A, p. 95.
3.	**List of glassware and chemicals**	x		Appendix A, pp. 90-91.
a.	**Are sources recommended?**	Chemicals	Glassware	
b.	**Are they commercially available?**	x		
4.	**Name, model, *etc.*, of the instrument, column, detector, *etc.*, used?**	x		Appendix A, pp. 90, 93.
a.	**Are sources recommended?**	x		
b.	**Are they commercially available?**	x		
5.	**LOD**			
a.	**Is there an explanation of how it was calculated?**	x		Appendix A, p. 97.
b.	**Is it a scientifically accepted procedure?**	x		
c.	**Is the matrix blank free of interference(s) at the retention time, wavelength, *etc.*, of the analyte(s) of interest?**	ILV (Figures 19-24, pp. 51-56)		ECM: Results from matrix blanks not reported.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
6.	**LOQ**			
a.	**Is there an explanation of how it was calculated?**		x	
b.	**Is it a scientifically accepted procedure?**			
7.	**Precision and accuracy data**			
a.	**Were there an adequate number of spiked samples analyzed?**			ECM: Number of fortified samples not reported.
b.	**Are the mean recoveries between 70-120%?**			ECM: Only results from one water sample fortified at the LOQ with all six compounds were reported.
c.	**Are the RSDs of the replicates 20% or less at or above the LOQ?**			
8.	**Description and/or explanation of:**			
a.	**Areas where problems may be encountered?**	x Only glass equipment may be used.		ECM stated water samples should not be collected or analyzed in plastic, as fluazinam and AMPA may adsorb (Appendix A, p. 92). ILV found all analytes adsorbed to polypropylene (p. 20).
b.	**Steps that are critical?**			None specified.
c.	**Interferences that may be encountered?**			None reported.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
9.	**Characterization of the Matrix(ces)?**		x	ECM: Source and characterization of the test water were not reported. ILV: Test (tap) water was not characterized.

Information obtained from p. 20; Figures 19-24, pp. 51-56; Appendix A, pp. 90-95, 97 of the study report.

V. Representative Chromatograms

		YES	NO	REVIEW FURTHER
A.	**Are there representative chromatograms for:**			
1.	**Analyte(s) in each matrix at the LOQ and 10 x LOQ?**	ECM: LOQ ILV: Both	ECM: 10 x LOQ	Figures 25-48, pp. 57-80; Appendix A, pp. 99-101.
2.	**Method blanks?**	ILV	ECM	Figures 13-18, pp. 45-50.
3.	**Matrix blanks?**	ILV	ECM	Figures 19-24, pp. 51-56.
4.	**Standard curves?**	ILV	ECM	Figures 1-6, pp. 33-38.
a.	**Do the standard curves have acceptable linearity?**	ILV		r = 0.9957-0.9994
5.	**Standards that can be used to recalculate some of the values for analyte(s) in the sample chromatograms?**	ILV	ECM	DER Attachment 2
B.	**Can the responses of the analytes(s) in the chromatograms of the lowest spiking level be accurately measured?**	x		Tables 7-11, pp. 27-31; Appendix A, pp. 99-101.

Information obtained from Tables 7-11, pp. 27-31; Figures 1-6, pp. 33-38; 13-48, pp. 45-80; Appendix A, pp. 99-101 of the study report.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

VI. Good Laboratory Practice (GLP) Standards

		YES	NO	REVIEW FURTHER
A.	**Is there a statement of adherence to the FIFRA GLP standards?**	ILV	ECM	

Information obtained from p. 3 of the study report.

VII. Independent Lab Validation (ILV)

		YES	NO	REVIEW FURTHER
A.	**Was an ILV performed?**	x		
B.	**Was the validation independent?**	x		p. 12.
C.	**Did the ILV's precision/accuracy data meet the criteria established in OCSPP Guideline 850.6100?**	x		
D.	**Were recommendations of major or minor modifications to the method made by the independent lab performing the ILV? If major modifications were suggested, what were they?**	x		There should be no substitutions for glass equipment (p. 20).

E. Recovery (Accuracy)/Precision Data; expressed as percentage of applied (n = 5)[1]

Spiking Level (µg a.i./L)	Parameter	Fluazinam	AMPA	DAPA	CAPA	DCPA	HYPA
0.10 (LOQ)	Range	85-87	110-113	96-102	107-114	96-115	109-123
	Mean	86	111	99	110	109	116
	SD	0.80	1.3	2.4	3.2	7.6	6.5
	RSD	0.94	1.2	2.4	2.9	7.0	5.7
1.0	Range	87-98	98-104	100-105	101-105	99-113	108-117
	Mean	94	101	103	102	106	112
	SD	4.2	2.3	1.8	1.6	6.5	3.9
	RSD	4.5	2.3	1.8	1.6	6.1	3.5

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

Information obtained from pp. 12, 20 of the study report. Shaded values are off acceptable limits.
1 Results obtained from Tables 1-6, pp. 23-26 of the study report; reported results verified by reviewer (DER Attachment 2). Recoveries from reagent blank samples fortified at 0.1 µg a.i./L were 95.0%, 99.0%, 75.9%, 85.9%, 42.5% and 64.4% for fluazinam, AMPA, DAPA, CAPA, DCPA and HYPA, respectively (Tables 7-12, pp. 27-32).
One step of sample transfer was saved by adding 0.2% formic acid in acetonitrile directly to the water (p. 20).

VIII. Completeness

		YES	NO	REVIEW FURTHER
A.	**Has enough information been supplied to do a proper review?**		x	See section ***IX. Recommendations*** (below).
B.	**Has enough information been supplied to do a laboratory evaluation, if requested?** [*BEAD ECB determination.*]			
C.	**Are all steps in the method scientifically sound?**	x		
D.	**Is a confirmatory method or technique provided?**	x		However, adequate supporting results were not provided.
E.	**Check the category below which best describes this ECM.**			
1.	**Satisfactory** [*Agency determination*]		x	Study is considered supplemental. Additional data should be provided to upgrade the studies.
2.	**Major Deficiencies**	x		See section ***IX. Recommendations*** (below).
3.	**Minor Deficiencies**	x		See section ***IX. Recommendations*** (below).

IX. Recommendations

1. For the ECM:

   a) Performance data were incomplete with only results from one water sample fortified at a nominal 0.1 µg a.i./L (LOQ) with all six compounds reported (Appendix A, pp. 99-101).

   b) The reported results could not be verified by the reviewer because supporting data were not provided.

   c) The number of fortified samples was not reported.

   d) Chromatograms of standards and method and matrix blank samples were not provided.

   e) Calibration curves and linear regression analyses were not provided.

   f) The source and characterization of the water matrix were not reported.

   g) No results from the confirmatory method were provided.

   h) No justification for selection of the LOQ concentration was provided.

   i) No regulatory guidelines were cited in the ECM (Appendix A, pp. 87-101).

2. For the ILV:

   a) For HYPA, two of the five fortifications at the LOQ (LOQ-1 121%, LOQ-5 123%) were not within acceptance criteria (*i.e.*, <70% or >120% recovery; EFED-ECM 2, Version 1, December 2010, p. 5; OCSPP 850.6100).

   b) Quantitative results from the confirmatory method were not reported and data on the representative chromatograms were illegible (Figures 49-54, pp. 81-86).

   c) Data on all chromatograms were for the most part illegible; therefore, verification of results using the chromatograms was done using Peak Area counts reported in Tables 7-12, pp. 27-32 of the study report (DER Attachment 2).

   d) The water matrix was not characterized, except for the source (tap).

Final Reviewer: José L. Meléndez
Date: 04/09/2013

José Luis Meléndez

Chemical: Fluazinam
PC: 129098
MRID: 48635802
Guideline: 850.6100

Independent laboratory validation for determination of fluazinam and its products AMPA, DAPA, CAPA, DCPA and HYPA in water.

Fortified (µg a.i./L)	Fluazinam					AMPA					DAPA				
	Measured (µg/L)	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Measured (µg/L)	Recovery (%)	Mean (%)	SD (%)	RSD (%)	Measured (µg/L)	Recovery (%)	Mean (%)	SD (%)	RSD (%)
0.1	0.0868	87				0.113	113				0.0979	98			
	0.0859	86				0.110	110				0.102	102			
	0.0847	85				0.110	110				0.0956	96			
	0.0853	85				0.111	111				0.100	100			
	0.0852	85	86	0.80	0.94	0.110	110	111	1.3	1.2	0.0999	100	99	2.4	2.4
1.0	0.922	92				1.01	101				1.02	102			
	0.870	87				0.976	98				1.00	100			
	0.975	98				1.01	101				1.05	105			
	0.955	96				1.00	100				1.03	103			
	0.959	96	94	4.2	4.5	1.04	104	101	2.3	2.3	1.03	103	103	1.8	1.8
Overall mean		90					106					101			
SD		5.1					5.6					2.7			
RSD		5.7					5.3					2.7			
Max		98					113					105			
Min		85					98					96			
n =		10					10					10			
Fortified (µg a.i./L)	CAPA					DCPA					HYPA				
	Measured (µg/L)	Recovery (%)	Mean (%)	SD (%)	RSD (%)	Measured (µg/L)	Recovery (%)	Mean (%)	SD (%)	RSD (%)	Measured (µg/L)	Recovery (%)	Mean (%)	SD (%)	RSD (%)
0.1	0.112	112				0.113	113				0.121	121			
	0.107	107				0.110	110				0.109	109			
	0.107	107				0.0957	96				0.109	109			
	0.112	112				0.110	110				0.116	116			
	0.114	114	110	3.2	2.9	0.115	115	109	7.6	7.0	0.123	123	116	6.5	5.7
1.0	1.02	102				1.11	111				1.17	117			
	1.02	102				1.07	107				1.11	111			
	1.01	101				0.991	99				1.08	108			
	1.01	101				0.993	99				1.15	115			
	1.05	105	102	1.6	1.6	1.13	113	106	6.5	6.1	1.09	109	112	3.9	3.5
Overall mean		106					107					114			
SD		4.9					6.8					5.4			
RSD		4.7					6.4					4.8			
Max		114					115					123			
Min		101					96					108			
n =		10					10					10			

Results (Calculated Concentration) from Tables 1-6, pp. 23-26 of the study report.
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
1 SD = Standard Deviation; determined using the “unbiased” or “n-1” method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.
Shaded cells mean they are off the recommended ranges.

Chemical: Fluazinam
PC: 129098
MRID: 48635802
Guideline: 850.6100

Verification of ILV recoveries in fortified water using chromatogram "Area" and calibration curve regression equations.

Fortified (µg a.i./L)	Analyte	Sample	Peak Area (counts)	Reviewer		Reported	
				Measured (µg/L)	Recovery (%)	Measured (µg/L)	Recovery (%)
0.1	Fluazinam	LOQ-1	15900	0.0871	87.1	0.0868	86.8
		LOQ-2	15700	0.0860	86.0	0.0859	85.9
	AMPA	LOQ-1	31700	0.113	113	0.113	113
		LOQ-2	30800	0.110	110	0.110	110
	DAPA	LOQ-1	15100	0.0982	98.2	0.0979	97.9
		LOQ-2	15600	0.102	102	0.102	102
	CAPA	LOQ-1	20100	0.113	113	0.112	112
		LOQ-2	18900	0.106	106	0.107	107
	DCPA	LOQ-1	4380	0.113	113	0.113	113
		LOQ-2	4270	0.110	110	0.110	110
	HYPA	LOQ-2	5650	0.109	109	0.109	109
		LOQ-3	5660	0.110	110	0.109	109
1.0	Fluazinam	LOQ-1	171000	0.922	92.2	0.922	92.2
		LOQ-2	162000	0.874	87.4	0.870	87.0
	AMPA	LOQ-1	323000	1.01	101	1.01	101
		LOQ-2	311000	0.974	97.4	0.976	97.6
	DAPA	LOQ-1	151000	1.03	103	1.02	102
		LOQ-2	147000	1.00	100	1.00	100
	CAPA	LOQ-1	196000	1.02	102	1.02	102
		LOQ-2	196000	1.02	102	1.02	102
	DCPA	LOQ-1	44200	1.11	111	1.11	111
		LOQ-2	42700	1.07	107	1.07	107
	HYPA	LOQ-1	51300	1.17	117	1.17	117
		LOQ-2	48500	1.11	111	1.11	111

Peak Area, Reported Measured (Calculated Concentration) and Reported Recovery (Accuracy) from Tables 7-12, pp. 27-32 for Figures 25-48, pp. 57-80 of the study report.

Linear regression coefficients from Figures 1-6, pp. 33-38 of the study report.

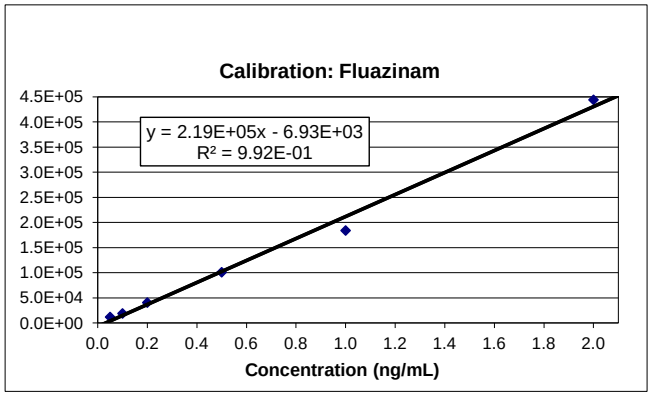
Measured calculated as using reported equations (p. 18).

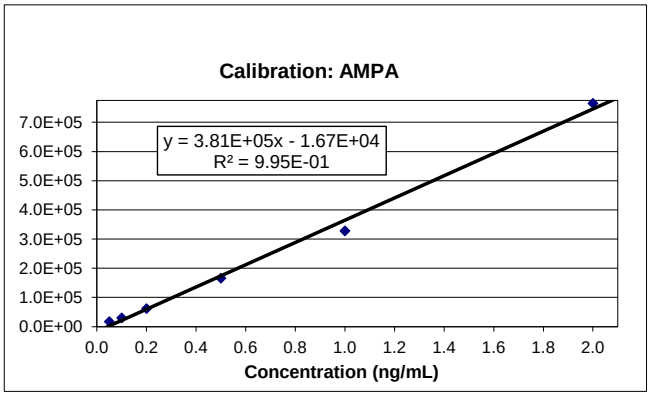
Chemical: Fluazinam
PC: 129098
MRID: 48635802
Guideline: 850.6100

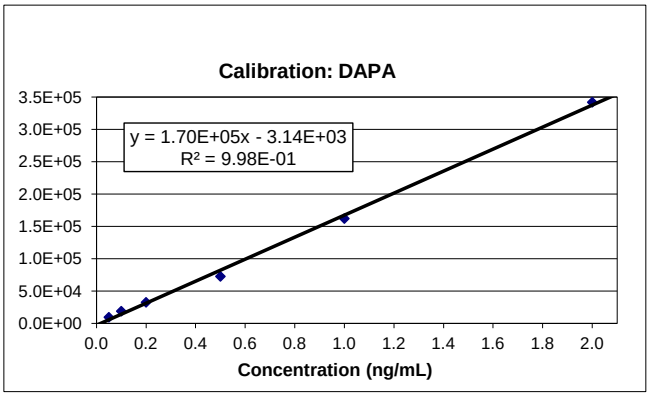
ILV calibration curves.

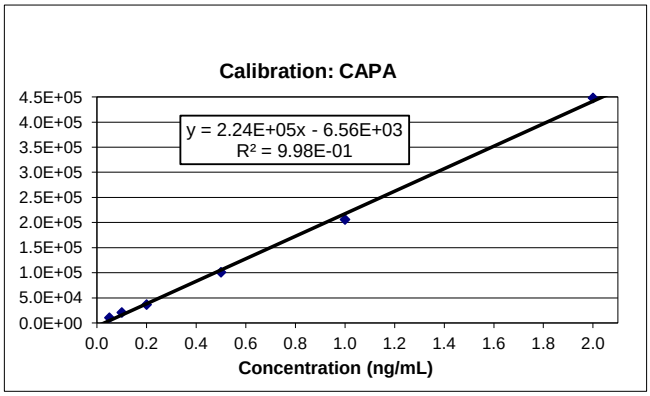
Concentration (ng/mL)	Fluazinam Peak Area (counts)	AMPA Peak Area (counts)	DAPA Peak Area (counts)	CAPA Peak Area (counts)	DCPA Peak Area (counts)	HYPA Peak Area (counts)
0.05	1.19E+04	1.71E+04	9.31E+03	1.05E+04	2.24E+03	3.66E+03
0.20	4.06E+04	6.17E+04	3.24E+04	3.66E+04	8.30E+03	9.63E+03
0.10	1.90E+04	2.99E+04	1.87E+04	2.09E+04	4.46E+03	
1.00	1.84E+05	3.28E+05	1.62E+05	2.06E+05	4.59E+04	4.60E+04
0.50	1.01E+05	1.66E+05	7.25E+04	1.01E+05	2.09E+04	2.50E+04
2.00	4.44E+05	7.65E+05	3.42E+05	4.48E+05	9.02E+04	1.01E+05

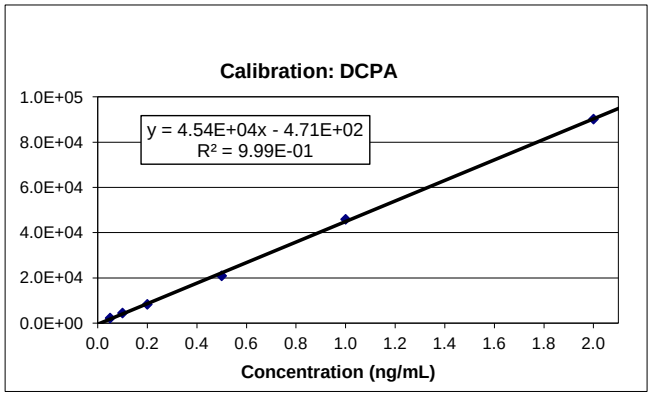
Results from Figures 1-6, pp. 33-38 of the study report.

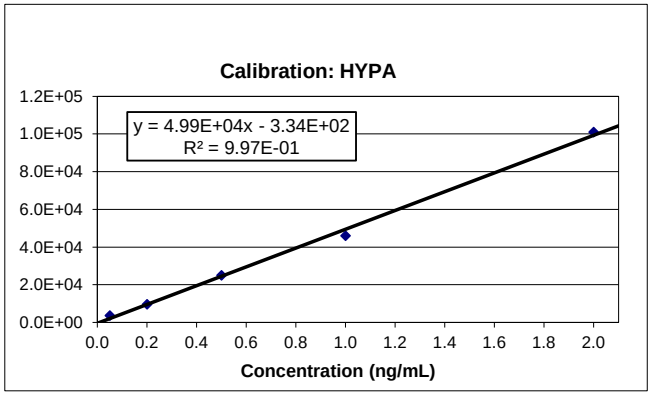












Chemical: Fluazinam
PC: 129098
MRID: 48635802
Guideline: 850.6100

ILV method (reagent) and matrix blank samples.

Analyte	Sample	Peak Area (counts)	Measured (µg/L)	Reported (µg/L)
Fluazinam	Reagent blank	1330	0.00865	N/A
	Matrix blank	667	0.00508	0.00508
	Matrix blank	0		No Peak
AMPA	Reagent blank	0		N/A
	Matrix blank	0		No Peak
	Matrix blank	0		No Peak
DAPA	Reagent blank	0		N/A
	Matrix blank	0		No Peak
	Matrix blank	0		No Peak
CAPA	Reagent blank	0		N/A
	Matrix blank	0		No Peak
	Matrix blank	0		No Peak
DCPA	Reagent blank	0		N/A
	Matrix blank	0		No Peak
	Matrix blank	0		No Peak
HYPA	Reagent blank	0		N/A
	Matrix blank	0		No Peak
	Matrix blank	0		No Peak

Peak Area and Reported (Calculated Concentration) from Tables 7-12, pp. 27-32 of the study report.
Linear regression coefficients from Figure 1, p. 33 of the study report.
Measured calculated as using reported equations (p. 18).