

Fluazinam; EPA PC Code 129098
ISK Biosciences Corporation; EPA Company Code
ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Test Material: Fluazinam

MRID: 48632402

Title: Karnik, S.C. 2011. Independent laboratory validation of enforcement method for the analysis of fluazinam in sediment.

MRID: 48632402 – Appendix A

Title: Robaugh, E. 2011. Method for the determination of fluazinam in sediment by LC/MS/MS.

EPA PC Code: 129098

OCSPP Guideline: 850.6100

For Cambridge Environmental

Primary Reviewer: Lynne Binari

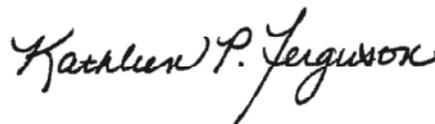
Signature:



Date: 5/14/12

Secondary Reviewer: Kathleen Ferguson

Signature:



Date: 5/14/12

QC/QA Manager: Joan Gaidos

Signature:



Date: 5/14/12

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Data Requirement: EPA Guideline: 835.6100
OECD Data Point: IIA 4.4

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 B, p. 40).

Primary Reviewer: *See signatures in cover page* **Date** *See above*
Cambridge Environmental

Final Reviewer:  **Date** 04/05/2013
U.S. EPA

ANALYTICAL METHOD: EPA MRID No. 48632402 – Appendix A. Robaugh, E. 2011. Method for the determination of fluazinam in sediment by LC/MS/MS. 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; 8 pages (p. 1A; Appendix A, pp. 32-39). Final report issued October 12, 2011 (Appendix A, p. 32).

INDEPENDENT LABORATORY VALIDATION: EPA MRID No. 48632402. Karnik, S.C. 2011. Independent laboratory validation of enforcement method for the analysis of fluazinam in sediment. 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; 51 pages. Final report issued October 18, 2011.

EXECUTIVE SUMMARY

This method is designed for the quantitative determination of residues of fluazinam in sediment using an external standardization method. The method was developed Pyxant Labs, for Ishihara Sangyo Kaisha, Ltd.; no regulatory guidelines were cited in the ECM (Appendix A, pp. 32-39). An independent laboratory validation (ILV), performed by Pyxant Labs Inc., was submitted with the method. The Agency finds that this method meets the criteria for a scientifically valid method and is **supplemental** for fluazinam in sediment. The registrant must provide the analytical results of the method validation.

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	48632402, Appendix A	48632402		Sediment	10/12/11	ISK Biosciences Corp.	LC/MS/MS-ESI ⁺¹	10 µg/kg ¹

1. Electrospray ionization in positive ion mode (ESI⁺)
2. The registrant must provide additional data regarding the environmental chemistry method. Available data is incomplete.

Method Summary: Fluazinam is extracted from sediment by sonication with methanol, the extract is diluted with water, then analyzed directly using LC/MS/MS (Appendix A, p. 33). The ECM defined a limit of quantitation (LOQ) of 0.01 mg/kg for fluazinam in sediment, which was supported by the ILV (Appendix A, pp. 37-38). A limit of detection (LOD) was not reported.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

For the ECM, performance data, LOD, chromatograms of standards and method and matrix blank samples, calibration curves and linear regression analyses, results from the confirmatory method, and the source and characterization of the sediment matrix were not provided.

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 (Table 1, p. 20). However, quantitative results from the confirmatory method were not reported and data on the representative chromatogram were illegible (Figure 10, p. 31).

ECM procedures specified that all QC samples could not be fortified at the same time, due to significant losses of analyte, consequently only two samples should be fortified and processed for analysis at a time (Appendix A, p. 36). However, the reported results

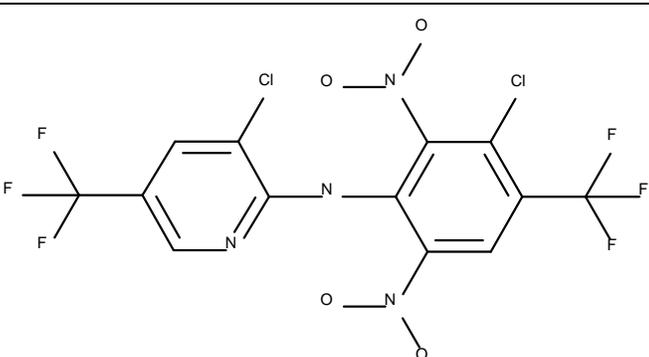
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indicate the ILV processed a batch of thirteen samples; one reagent blank, two unfortified control samples, five samples fortified at the LOQ, and five samples fortified at 10 x LOQ (pp. 12-13).

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. 40).
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.
CAS #	79622-59-6
Structure	

Information obtained from p. 1A; Appendix B, p. 40 of the study report. CAS name and compound structure obtained from Fluazinam structures[1].doc.

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound	
Parameter	Value
Melting point/range (°C)	Not reported.
pH	Not reported.
Density (g/cm ³)	Not reported.
Water solubility at 20°C (mg/L)	Not reported.

Parameter	Value
Solvent solubility at 20 °C (mg/L)	Not reported.
Vapor pressure at ___ °C (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

Sediment is extracted by sonication with methanol, then the extract is diluted with water and analyzed directly for fluazinam by LC/MS/MS-ESI⁺ using a Phenomenex Synergi Polar-RP column (Appendix A, pp. 36-37). Two ion transitions are monitored for quantitation and confirmation.

Parameter	Value
Method ID	Method for the determination of fluazinam in sediment by LC/MS/MS. Pyxant Labs Inc. Method Number STM2360.03 (Appendix A, p. 32).
Analyte(s)	Fluazinam.
Extraction solvent/technique	Sediment (10 g) is transferred to a glass tube, extracted once with methanol (30 mL) using a digital sonifier, with cup horn, for 10 minutes; pulse signal 50 seconds on/10 seconds off/80% capacity (Appendix A, p. 36).
Cleanup strategies	Extract and sediment separated by centrifugation, then extract diluted 1:10 (v:v) with water for analysis (Appendix A, pp. 36-37).
Instrument/Detector	<u>ECM</u> : Symbiosis HPLC system with Phenomenex Synergi Polar-RP column (2 x 50 mm, 4-µm) and Applied Biosystems API5000 LC/MS/MS equipped with Turbo Spray electrospray ionization in positive ion mode (ESI ⁺) and multiple reaction monitoring (MRM; Appendix A, p. 37). <u>ILV</u> : same as ECM except Shimadzu HPLC system (pp. 14, 18).

Information obtained from pp. 14, 18; Appendix A, pp. 32, 36-37 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 in Sediment			
Analyte	Spiking Level (mg a.i./kg)	Recoveries Obtained (%)	Relative Standard Deviation
Fluazinam	0.01 (LOQ)	--	--
	0.1	--	--

Results (Spiking Level) from Appendix A, pp. 37-38 of the study report.
 -- = Not reported.

Results from the confirmatory method were not reported.

C.1.1. Method Characteristics

TABLE C.2. Method Characteristics	
Parameter	Value
Analyte(s)	Fluazinam.
Limit of Quantitation (LOQ)	0.01 mg a.i./kg (Appendix A, pp. 37-38).
Limit of Detection (LOD)	Not reported.
Accuracy/Precision at LOQ	<u>ECM</u> : Performance data were not reported.
Reliability of the Method/[ILV]	<u>ILV</u> : Acceptance criteria were met at the LOQ with matrix spike recoveries ranging between 70% to 120% and relative standard deviations of ≤20% (Table 1, p. 20). The method was validated in one trial (p. 17).
Linearity	<u>ECM</u> : not reported. <u>ILV</u> : Linear regression: $r = 0.99821$ (Figure 1, p. 22).
Specificity	<u>ECM</u> : could not be determined because quantitative results and chromatograms for standards and method and matrix blank samples were not provided. <u>ILV</u> : 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 (Figures 2-9, pp. 23-30). Method and matrix blank controls showed no significant interferences at the retention time of the analyte (Tables 1-2, pp. 20-21; Figures 4-6, pp. 25-27).

Information obtained from p. 17; Tables 1-2, pp. 20-21; Figure 1, p. 22; Appendix A, pp. 37-38 of the study report.

C.2. Independent Laboratory Validation (ILV)

The ILV was conducted in compliance with USEPA GLP Standards 40 CFR, Part 160, OPPTS 850.7100 guidelines, and PR Notice 96-1 (pp. 3, 10, 19).

Analyte	Spiking Level (mg a.i./kg)	Mean Recoveries Obtained (%)	Relative Standard Deviation
Fluazinam	0.01 (LOQ)	83	9
	0.1	96	3

Results obtained from Table 1, p. 20; reported results verified by reviewer (DER Attachment 2).

Quantitative results from the confirmatory method were not reported and data on the representative chromatogram were illegible (Figure 10, p. 31).

D. CONCLUSION

This method is designed for the quantitative determination of residues of fluazinam in sediment. The Agency finds that this method meets the criteria for a scientifically valid method and is **supplemental** for fluazinam in sediment. The registrant must provide the analytical results of the validation of the method.

[Refer to the review checklist attached to this review.]

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	Method for the determination of fluazinam in sediment by LC/MS/MS (Appendix A, p. 32). Pyxant Labs Inc. Method Number STM2360.03.	
B.	ECM No. [ECB use]		
C.	MRID No.	48632402	
D.	Matrix	Sediment	
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. 40).
		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. 40).

Information obtained from p. 1A; Appendix A, p. 32; Appendix B, p. 40 of the study report. CAS name and compound structure obtained from Fluazinam structures[1].doc.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

II. Information about the Laboratory

A.	Name	Pyxant Labs Inc. (Appendix A, p. 32).
B.	Address	4720 Forge Road, Suite 108, Colorado Springs, Colorado 80907.
C.	Telephone No.	719-593-1165
D.	Name of the Study Director	Not reported.
E.	Name of the Lead Chemist	Erin Robaugh.
F.	Laboratory Validation:	Not provided.

Information obtained from Appendix A, p. 32 of the study report.

III. Method Summary Information for Analyte(s): Fluazinam.

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.
B.	Sample Preparation	Sediment (10 g) was fortified with a standard solution of fluazinam, in acetonitrile, at 0.01 and 0.1 mg a.i./kg (Appendix A, pp. 35-38). Application solution volumes were not reported.
C.	Sample Extraction	Sediment (10 g) extracted once with methanol (30 mL) using a digital sonifier, with cup horn, for 10 minutes; pulse signal 50 seconds on/10 seconds off/80% capacity (Appendix A, p. 36).
D.	Sample Cleanup	Extract and sediment separated by centrifugation and decanted. An aliquot of the extract is diluted 1:10 (v:v) with water for analysis (Appendix A, p. 37).
E.	Sample Derivatization (if applicable)	None reported.
F.	Sample Analysis	LC/MS/MS (Appendix A, p. 33).

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

1.	Instrumentation	Symbiosis Pharma HPLC System and Applied Biosystems API5000 LC/MS/MS equipped with Turbo Spray electrospray ionization in positive ion mode (ESI ⁺ ; Appendix A, p. 37).			
2.	Primary Column	Phenomenex Synergi Polar-RP column (2 x 50 mm, 4 μm) with Phenomenex C18 guard column (optional; Appendix A, p. 37).			
3.	Confirmatory Column (if any)	None reported.			
4.	Detector	Multiple Reaction Monitoring (MRM; Appendix A, p. 37).			
5.	Other Confirmatory Techniques (if any)	Two ion transitions were monitored for quantitation and confirmation (Appendix A, p. 37).			
6.	Other Relevant Information	Compound	Ions monitored (m/z)		Retention time (min.) ¹
			Quantitation	Confirmation	
		Fluazinam	465.2 > 373.0	465.2 > 338.0	ca. 5.20
G.	Detection and Quantitation Limits				
1.	Limit of Quantitation (LOQ)				
	Claimed in Method				0.01 mg/kg (Appendix A, p. 38).
2.	Limit of Detection (LOD)				
	Claimed in Method				Not reported.
H.	Recovery (Accuracy)/Precision Data; expressed as percentage of applied				
	Spiking Level (mg a.i./kg)	Parameter	Fluazinam		
	0.01 (LOQ)	Range	--		
		Mean	--		
		SD	--		
		RSD	--		
	0.1	Range	--		
		Mean	--		
SD		--			
RSD		--			

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

Information obtained from pp. 2, 14; Appendix A, pp. 33, 35-38 of the study report.

-- = Not reported.

1 Obtained from ILV (p. 14); HPLC retention time not reported in ECM.

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. 36.			
B.	If the method requires explosive or carcinogenic reagents, are proper precautions explained?			Not applicable.			
C.	Is the following information supplied?						
1.	Detailed stepwise description of:						
a.	The sample preparation procedure?				x		Appendix A, p. 36.
b.	The sample spiking procedure?					x	
c.	The extraction procedure?				x		Appendix A, p. 36.
d.	The derivatization procedure?						Not applicable.
e.	The clean-up procedure?				x		Appendix A, p. 36.
f.	The analysis procedure?				x		Appendix A, p. 37.
2.	Procedures for:						
a.	Preparation of standards?				x		Appendix A, p. 36.
b.	Calibration of instrument?	x		Appendix A, pp. 37-38.			
3.	List of glassware and chemicals	x		Appendix A, pp. 33-34.			
a.	Are sources recommended?	Extraction tubes, HPLC vials	Chemicals, Additional glassware				
b.	Are they commercially available?	x					
4.	Name, model, etc., of the instrument, column, detector, etc., used?	x		Appendix A, pp. 34, 37.			
a.	Are sources recommended?	x					

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
b.	Are they commercially available?	x		
5.	LOD			
a.	Is there an explanation of how it was calculated?		x	LOD not reported.
b.	Is it a scientifically accepted procedure?			
c.	Is the matrix blank free of interference(s) at the retention time, wavelength, etc., of the analyte(s) of interest?			ECM: Results from matrix blanks not reported.
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: No performance data.
b.	Are the mean recoveries between 70-120%?			
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?			All QC fortifications cannot be prepared at the same time or significant loss of analyte occurs (Appendix A, p. 36). Only two QC samples are fortified and processed for analysis at a time.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
b.	Steps that are critical?			Analysis must be performed as quickly as possible (Appendix A, p. 36).
c.	Interferences that may be encountered?			None reported.
9.	Characterization of the Matrix(ces)?	ILV	ECM	p. 11.

Information obtained from p. 11; Appendix A, pp. 33-34, 36-38 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?	ILV	ECM	Figures 6-9, pp. 27-30.
2.	Method blanks?	ILV	ECM	Figure 4, p. 25.
3.	Matrix blanks?	ILV	ECM	Figure 5, p. 26.
4.	Standard curves?	ILV	ECM	Figure 1, p. 22.
a.	Do the standard curves have acceptable linearity?	x		r = 0.99821
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		Table 2, p.21.

Information obtained from Table 2, p. 21; Figure 4, p. 25; Figure 1, p. 22; Figures 4-9, pp. 25-30 of the study report.

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EPA MRID Number 48632402 (ECM/ILV)

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.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

VII. Independent Lab Validation (ILV)

		YES	NO	REVIEW FURTHER
A.	Was an ILV performed?	x		
B.	Was the validation independent?	x		The ILV was reported as being conducted in a different department and by personnel who had no prior experience or knowledge of the ECM procedures (pp. 9-10).
C.	Did the ILV's precision/accuracy data meet the criteria established in OPPTS Guideline 850.7100?	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		Minor (p. 18).
E.	Recovery (Accuracy)/Precision Data; expressed as percentage of applied (n = 5)¹			
	Spiking Level (mg a.i./kg)	Parameter	Fluazinam	
	0.01 (LOQ)	Range	73-94	
		Mean	83	
		SD	8	
		RSD	9	
	0.1	Range	91-99	
		Mean	96	
SD		3		
RSD		3		

Information obtained from pp. 9-10, 18 of the study report.

¹ Results obtained from Table 1, p. 20 of the study report; reported results verified by reviewer (DER Attachment 2).

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

VIII. Completeness

		YES	NO	REVIEW FURTHER
A.	Has enough information been supplied to do a proper review?		x	See section <i>IX. Recommendations</i> (below).
B.	Has enough information been supplied to do a laboratory evaluation, if requested? [<i>This may be left blank, as it is a determination made by BEAD ECB.</i>]			
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 [<i>Agency determination</i>]		x	
2.	Major Deficiencies	x		See section <i>IX. Recommendations</i> (below).
3.	Minor Deficiencies	x		See section <i>IX. Recommendations</i> (below).

IX. Recommendations

1. For the ECM:
 - a) No performance data were provided.
 - b) No chromatograms were provided.
 - c) The limit of detection was not reported.
 - d) The source and characterization of the sediment matrix were not reported.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

- e) HPLC retention time for the analyte was not reported.
 - f) No results from the confirmatory method were provided.
 - g) No justification for selection of the LOQ concentration was provided.
 - h) No regulatory guidelines were cited in the ECM (Appendix A, pp. 32-39).
2. For the ILV:
- a) Quantitative results from the confirmatory method were not reported and data on the representative chromatogram were illegible (Figure 10, p. 31).
 - b) 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 Table 2, p. 21 of the study report (DER Attachment 2).

Cambridge Environmental see signatures in the cover page of the DER

Primary Reviewer



04/05/2013

Secondary Reviewer: José L. Meléndez, Chemist

Chemical: Fluazinam

PC: 129098

MRID: 48632402

Guideline: 850.7100

Independent laboratory validation for determination of fluazina

Fortified (mg a.i./kg)	Fluazinam				
	Measured (mg/kg)	Recovery (%)	Mean (%)	SD ¹ (%)	RSD ² (%)
0.01	0.00933	93			
	0.00726	73			
	0.00864	86			
	0.00789	79			
	0.00834	83	83	8	9
0.1	0.0942	94			
	0.0990	99			
	0.0987	99			
	0.0948	95			
	0.0912	91	96	3	3
Overall mean		89			
SD		9			
RSD		10			
Max		99			
Min		73			
n =		10			

Chemical: Fluazinam

PC: 129098

MRID: 48632402

Guideline: 850.7100

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

Fortified (mg a.i./kg)	Analyte	Sample	Peak Area (counts)	Reviewer		Reported	
				Measured (mg/kg)	Recovery (%)	Measured (mg/kg)	Recovery (%)
0.01	Fluazinam	LOQ-1	24200	0.00934	93	0.00933	94
		LOQ-2	18900	0.00725	72	0.00726	73
0.1		LOQ-1	240000	0.0943	94	0.0942	94
		LOQ-2	252000	0.0990	99	0.0990	99

Peak Area from Table 2, p. 21 for Figures 6-9, pp. 27-30 of the study report.

Linear regression coefficients from p. 16; Figure 1, p. 22 of the study report.

Reported Measured and Recovery from Table 1, p. 20 of the study report.

Measured calculated as using reported equations (pp. 15-16).

Chemical: Fluazinam
PC: 129098
MRID: 48632402
Guideline: 850.7100
ILV calibration curve.

Concentration (ng/mL)	Fluazinam	
	Peak Area (counts)	
0.05	6.26E+03	
0.10	7.53E+03	
0.20	1.17E+04	
0.50	3.55E+04	
1.00	6.94E+04	
2.00	1.50E+05	
10.0	7.79E+05	

Results from Table 2, p. 21 of the study report.

