

Test Material: Pyriofenone

MRID: 49256133

Title: IKF-309: VALIDATION OF METHODOLOGY FOR THE DETERMINATION OF RESIDUES IN SURFACE AND DRINKING WATER

MRID: 49321801

Title: Independent Laboratory Validation of Ishihara Sangyo Kaisha (ISK) Residue Analytical Method for IKF-309 Determination of Residues in Surface and Drinking Water (Document Number: JSM0058)

EPA PC Code: 028828

OCSP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lynne Binari

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Date: 11/3/14

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QC/QA Manager: Joan Gaidos

Signature: 

Date: 11/3/14

Analytical method for pyriofenone (IKF-309) in water

Reports: ECM: EPA MRID No.: 49256133. Airs, D. 2010. IKF-309: VALIDATION OF METHODOLOGY FOR THE DETERMINATION OF RESIDUES IN SURFACE AND DRINKING WATER. Document No.: JSM0058. Report prepared by Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, sponsored by ISHIHARA SANGYO KAISHA, LTD., Osaka, Japan, and submitted by ISK BIOSCIENCES CORPORATION, Painesville, Ohio; 50 pages, plus three front pages 1A-1C. Final report issued March 4, 2010.
ILV: EPA MRID No. 49321801. Schoenau, E. 2014. Independent Laboratory Validation of Ishihara Sangyo Kaisha (ISK) Residue Analytical Method for IKF-309 Determination of Residues in Surface and Drinking Water (Document Number: JSM0058). GPL Study No.: 140531 and Report No.: IB-2014-JLW-007-01-01. 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; 115 pages. Final report issued March 19, 2014.

Document No.: MRIDs 49256133 & 49321801

Guideline: 850.6100
EC SANCO/3029/99 rev. 4 & SANCO/825/00 rev. 7 (p. 9 of MRID 49256133)

Statements: ECM: The study was conducted in compliance with UK Good Laboratory Practice (GLP) Regulations, OECD Principles of GLP and EC Commission Directive 2004/10/EC, which are compatible with USEPA GLP standards (pp. 1C, 2 of MRID 49256133). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 1B-1C, 2-3, 50). An Authenticity Certification statement was not provided.
ILV: The study was conducted in compliance with USEPA GLP standards, with the exception of the surface water characterization (p. 3 of MRID 49321801). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statement were provided (pp. 2-4). A statement of the authenticity of the study report was included as part of the Quality Assurance Statement (p. 4). A signature page was included (p. 5).

Classification: This analytical method is classified as **acceptable**. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The drinking water matrices were not characterized. However, the LOQ is orders of magnitude less than the lowest toxicological level of concern in water.

PC Code: 028828

Reviewer: Faruque Khan
Senior Scientist

Signature:
Date: December 5, 2014

Executive Summary

This analytical method, DETERMINATION OF RESIDUES OF IKF-309 IN WATER (Appendix 3, p. 47 of MRID 49256133), is designed for the quantitative determination of pyriofenone (IKF-309) in water using HPLC/MS/MS. The method is quantitative for pyriofenone at the stated LOQ of 0.05 µg/L. The LOQ is orders of magnitude less than the lowest toxicological level of concern in water. The independent laboratory validated the method for analysis of pyriofenone in surface water and drinking water after one trial. No major modifications were made by the independent laboratory. For both the ECM and ILV, the drinking water matrices were not characterized.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix ¹	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Pyriofenone (IKF-309)	49256133	49321801		Surface water and drinking water	04/03/2010	ISK Biosciences Corporation	HPLC/MS/MS	0.05 µg/L

¹ For both the ECM and ILV, the surface water matrices were characterized, but not the drinking water matrices (p. 10 of MRID 49256133; pp. 16-17 of MRID 49321801).

I. Principle of the Method

Water (10 mL) was combined with 10 mL acetonitrile, further diluted with acetonitrile:water (50:50, v:v) if required, then analyzed directly by LC/MS/MS (Appendix 3, p. 48 of MRID 49256133).

Samples were analyzed for pyriofenone (IKF-309) by HPLC (Phenomenex Luna C₈, 2 mm x 15 cm column) using a mobile phase of (A) water:methanol:formic acid (90:100.1, v:v:v) containing 0.01M ammonium formate and (B) methanol:formic acid (100:0.1, v:v) [percent A:B (v:v) at 0 min. 30:70, 6-10 min. 0:100, 11-15 min. 30:70] with MS/MS-ESI (Quattro LC/Varian 1200/Acquity TQD system, electrospray ionization, positive ion mode) detection and multiple reaction monitoring (MRM), also called selected reaction monitoring (SRM; p. 15; Appendix 3, p. 49 of MRID 49256133). Injection volume was 20 µL. Pyriofenone was identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 366→184 (Q) and *m/z* 366→209 (C). Pyriofenone retention time was *ca.* 5.5 minutes.

The ILV performed the method as written with equivalent instrumentation substitutions (pp. 12-14, 16; Appendix B, p. 44 of MRID 49321801). An AB Sciex API 4000 LC/MS/MS was utilized (pp. 17-18). Pyriofenone retention time was 6.2 minutes.

The LOQ and LOD for pyriofenone in water were the same in the ECM and ILV at 0.05 µg/L (ppb) and 0.01 ng/mL (equivalent to 0.02 µg/L in the sample matrix), respectively (p. 14; Appendix 3, p. 49 of MRID 49256133; p. 23 of MRID 49321801).

II. Recovery Findings

ECM (MRID 49256133: Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of pyriofenone (IKF-309) at fortification levels of 0.05 $\mu\text{g/L}$ (LOQ) and 0.5 $\mu\text{g/L}$ (10x LOQ) in surface water and drinking water (p. 14). Pyriofenone was identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The surface water matrix was characterized, but not the drinking water (p. 10).

ILV (MRID 49321801): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of pyriofenone in surface water and drinking water at fortification levels of 0.05 $\mu\text{g/L}$ (LOQ) and 0.5 $\mu\text{g/L}$ (10x LOQ; p. 12). Pyriofenone was identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The method was validated for pyriofenone in the two water matrices at both fortification levels after one trial, with no method modifications (pp. 12, 20). The surface water matrix was characterized, but not the drinking water (pp. 16-17).

Table 2. Initial Validation Method Recoveries for Pyriofenone (IKF-309) in Water

Matrix ¹	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water	Quantitation ion					
	0.05 (LOQ)	5	102-110	107	3.4	3.2
	0.5	5	102-104	103	0.8	0.8
	Confirmation ion					
	0.05 (LOQ)	5	85-109	102	9.7	9.5
	0.5	5	97-102	100	2.3	2.3
Drinking Water	Quantitation ion					
	0.05 (LOQ)	5	100-110	105	4.3	4.1
	0.5	5	102-106	104	1.5	1.5
	Confirmation ion					
	0.05 (LOQ)	5	89-109	103	8.2	7.9
	0.5	5	100-109	104	3.7	3.5

Data (uncorrected recovery results) were obtained from Tables 3-6, pp. 18-21 of MRID 49256133 and DER Attachment 2 (standard deviations).

¹ The surface water was obtained from local source Costessey Pit No. 1, and characterization was provided (p. 10 of MRID 49256133). The drinking water was obtained within Huntingdon Life Sciences Environmental Analysis Department, but a matrix characterization was not provided.

Table 3. Independent Validation Method Recoveries for Pyriofenone (IKF-309) in Water

Matrix ¹	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water	Quantitation ion					
	0.05 (LOQ)	5	109-114	111	2.00	1.80
	0.5	5	104-107	106	1.34	1.26
	Confirmation ion					
	0.05 (LOQ)	5	108-111	109	1.14	1.05
	0.5	5	105-107	106	0.837	0.790
Drinking Water	Quantitation ion					
	0.05 (LOQ)	5	108-113	110	1.82	1.65
	0.5	5	104-107	106	1.14	1.08
	Confirmation ion					
	0.05 (LOQ)	5	101-112	105	4.55	4.33
	0.5	5	104-106	105	0.837	0.797

Data (uncorrected recovery results) were obtained from Tables I-IV, pp. 26-29 of MRID 49321801.

¹ The surface water was obtained from Fresno Irrigation District Herndon Canal No. 39 near the Gates Avenue Bridge on February 25, 2014, and characterization was provided (pp. 16-17 of MRID 49321801). The drinking water was obtained within Golden Pacific Laboratories on March 11, 2014, but a matrix characterization was not provided.

III. Method Characteristics

The LOQ and LOD of 0.05 µg/L and 0.01 ng/mL (equivalent to 0.02 µg/L in sample matrix), respectively, for pyriofenone in water were the same in the ECM and ILV (p. 14 of MRID 49256133; p. 23 of MRID 49321801). The ECM defined the LOQ as the lowest fortification level at which acceptable recovery data were obtained. The ECM defined the LOD as the concentration of the lowest calibration standard chromatographed.

Table 4. Method Characteristics for Pyriofenone (IKF-309) in Water

		Pyriofenone
Limit of Quantitation (LOQ)		0.05 µg/L
Limit of Detection (LOD)		0.01 ng/mL (equivalent to 0.02 µg/L in sample matrix)
Linearity (no weighting, calibration curve r^2 and concentration range) ¹	ECM:	Q ion: $r^2 = 0.9998$ C ion: $r^2 = 0.9996$
	ILV:	Q ion: $r^2 = 0.9998-1.0000$ C ion: $r^2 = 0.9996-0.9998$
	Range:	0.01-1 ng/mL
Repeatable		Yes
Reproducible		Yes
Specific		Yes

Data were obtained from p. 14; Tables 1-2, p. 17 of MRID 49256133; p. 23; Appendix D, pp. 92-95 of MRID 49321801.

¹ Linearity of the provided ECM and ILV calibration curves was verified by the reviewer (DER Attachment 2).

IV. Method Deficiencies and Reviewer's Comments

- The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. The LOQ and LOD of 0.05 µg/L

and 0.01 ng/mL (equivalent to 0.02 µg/L in sample matrix), respectively, for pyriofenone in water were the same in the ECM and ILV (p. 14 of MRID 49256133; p. 23 of MRID 49321801). The ECM defined the LOQ as the lowest fortification level at which acceptable recovery data were obtained. The ECM defined the LOD as the concentration of the lowest calibration standard chromatographed.

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

2. For both the ECM and ILV, characterization of the drinking water matrices was not provided (p. 10 of MRID 49256133; pp. 16-17 of MRID 49321801).
3. For the ILV, documentation for communication between the independent laboratory and the developers and/or previous users of the ECM was not presented in the study report.
4. For the ECM, chromatograms of reagent blank samples were not provided. For matrix blank control samples, no interferences (i.e. <30% of LOQ) at the retention time of pyriofenone (IKF-309) were observed for either ion transition (pp. 8, 14; Figure 6, p. 28; Figure 9, p. 31 of MRID 49256133). In addition, no significant enhancement or suppression of response (matrix effects) was observed for pyriofenone in the final sample extracts (p. 15; Appendix 2, pp. 39-42).
5. As part of the ECM, pyriofenone, at 1 µg/L (ng/mL), in final extracts of surface water and drinking water was found to be stable when stored at -20°C for 6 days with recoveries of 95-104% (pp. 11, 15; Table 7, p. 22 of MRID 49256133).
6. It was reported for the ILV that one analyst could prepare a sample set in one hour, with overnight LC/MS/MS analysis, followed by an additional 0.5 hour for data calculation and tabulation (p. 20 of MRID 49321801). Therefore, two calendar days are required for sample preparation, analysis, and calculation/tabulation of the data.

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.

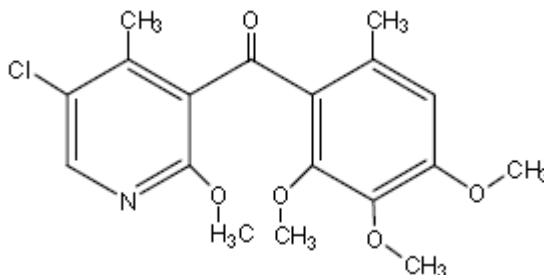
Attachment 1: Chemical Names and Structures**Pyriofenone (IKF-309)**

IUPAC Name: (5-Chloro-2-methoxy-4-methyl-3-pyridyl)(2,3,4-trimethoxy-6-methylphenyl)ketone

CAS Name: (5-Chloro-2-methoxy-4-methyl-3-pyridinyl)(2,3,4-trimethoxy-6-methylphenyl)methanone

CAS Number: 688046-61-9

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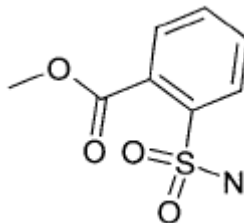
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IUPAC Name:

CAS Name:

CAS Number:

SMILES String:



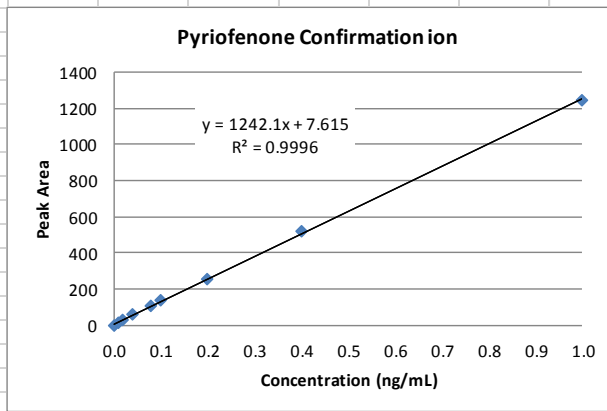
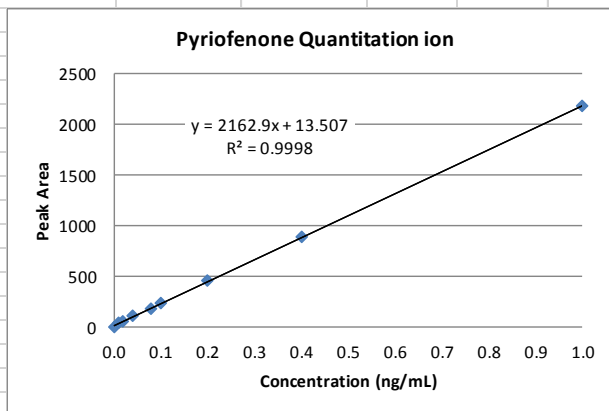
Attachment 2. Calculations

Chemical: Pyriofenone														
PC: 028828														
MRIDs: 49256133 (ECM) & 49321801 (ILV)														
Guideline: 850.6100														
ECM Validation for Determination of Pyriofenone (IKF-309) in Water														
Fortified (µg a.i./L)	Surface (Costessey Pit No. 1) Water							Drinking (Huntingdon Life Sciences) Water						
	Recovery (%)	Mean (%)	SD ¹ (%)	RSD ² (%)	Max	Min	n =	Recovery (%)	Mean (%)	SD ¹ (%)	RSD ² (%)	Max	Min	n =
Quantitation m/z 366 > 184														
0.05 LOQ	109							108						
	102							110						
	110							104						
	104							100						
	108	107	3.4	3.2	110	102	5	101	105	4.3	4.1	110	100	5
0.5	103							105						
	104							106						
	102							105						
	104							104						
	103	103	0.8	0.8	104	102	5	102	104	1.5	1.5	106	102	5
Confirmation m/z 366 > 209														
0.05 LOQ	106							108						
	105							104						
	85							89						
	106							109						
	109	102	9.7	9.5	109	85	5	106	103	8.2	7.9	109	89	5
0.5	101							104						
	102							109						
	97							100						
	102							106						
	98	100	2.3	2.3	102	97	5	101	104	3.7	3.5	109	100	5
Results from Tables 3-6, pp. 18-21 of MRID 49256133. The surface water was characterized, but not the drinking water (p. 10). 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.														

Chemical: Pyriofenone
 PC: 028828
 MRIDs: 49256133 (ECM) & 49321801 (ILV)
 Guideline: 850.6100
 ECM Calibration Curves

Concentration (ng/mL)	Pyriofenone (IKF-309)	
	Quantitation Peak Area Counts	Confirmation Peak Area Counts
0	0	0
0.01	32.945	10.827
0.02	51.905	28.215
0.04	111.173	59.140
0.08	182.147	110.326
0.1	229.393	135.984
0.2	462.158	258.668
0.4	880.829	521.501
1	2172.417	1241.850

Results (Peak Area Counts) from Tables 1-2, p. 17 of MRID 49256133.



Chemical: Pyriofenone
 PC: 028828
 MRIDs: 49256133 (ECM) & 49321801 (ILV)
 Guideline: 850.6100
 ECM Calibration Curves

Concentration (ng/mL)	Pyriofenone (KF-309)			
	Surface Water Sample Sets		Drinking Water Sample Sets	
	Quantitation	Confirmation	Quantitation	Confirmation
	Peak Area Counts	Peak Area Counts	Peak Area Counts	Peak Area Counts
0.01	3487.1	2656.1	3510.6	2668.8
0.02	7218.0	4558.5	6481.2	4595.5
0.04	14237.2	9825.1	13777.4	9518.2
0.08	27836.2	19014.5	27239.9	18211.3
0.1	37337.5	25423.1	34724.5	24404.7
0.2	74917.9	51590.7	72757.0	49784.3
0.4	151624.6	104649.8	146302.2	99681.0
0.8	300988.1	202122.0	286401.4	192465.1
1	380852.6	260205.6	361962.6	245145.6

Results (Peak Area Counts) from Appendix D, pp. 92-95 of MRID 49321801.

