| | |
|---|---|
| **Test Material:** | Pyriofenone |
| **MRID:** | 49256126 |
| **Title:** | VALIDATION OF A RESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF IKF-309 IN AGRICULTURAL SOIL |
| **MRID:** | 49256127 |
| **Title:** | INDEPENDENT LABORATORY VALIDATION (ILV) OF THE RESIDUE ANALYTICAL METHOD FOR DETECTION OF IKF-309 IN SOIL (RCC STUDY #B18843) |
| **EPA PC Code:** | 028828 |
| **OCSPP Guideline:** | 850.6100 |

**For CDM Smith**

**Primary Reviewer:** Lynne Binari          **Signature:**

**Date:** 11/3/14

**Secondary Reviewer:** Lisa Muto          **Signature:**

**Date:** 11/3/14

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

**Date:** 11/3/14

**Analytical method for pyriofenone (IKF-309) in soil**

| | |
|---|---|
| **Reports:** | ECM: EPA MRID No.: 49256126. Wolf, S. 2007. VALIDATION OF A RESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF IKF-309 IN AGRICULTURAL SOIL. RCC Study No.: B18843. Report prepared by RCC Ltd., Itingen, Switzerland, sponsored by ISHIHARA SANGYO KAISHA, LTD., Osaka, Japan, and submitted by ISK BIOSCIENCES CORPORATION, Concord, Ohio; 87 pages, plus three front pages 1A-1C. Final report issued July 6, 2007. |
| | ILV: EPA MRID No. 49256127. Ferguson, L.-J. 2011. INDEPENDENT LABORATORY VALIDATION (ILV) OF THE RESIDUE ANALYTICAL METHOD FOR DETECTION OF IKF-309 IN SOIL (RCC STUDY #B18843). Ricerca Study No.: 027336 and Document No.: 027336-1 (p. 1). ISKBC Document No.: IB-2011-JLW-022-01. Report prepared by Ricerca Biosciences, LLC, Concord, Ohio, sponsored by ISHIHARA SANGYO KAISHA, LTD., Osaka, Japan, and submitted by ISK BIOSCIENCES CORPORATION, Concord, Ohio; 154 pages, plus three front pages 1A-1C. Final report issued July 21, 2011. |
| **Document No.:** | MRIDs 49256126 & 49256127 |
| **Guideline:** | 850.6100 |
| | EC SANCO/3029/99 rev. 4 & SANCO/825/00 rev. 7 (p. 11 of MRID 49256126) |
| | OPPTS 860.1340, OPPTS 850.7100, & EC SANCO/825/00 rev. 8 (p. 6 of MRID 49256127) |
| **Statements:** | ECM: The study was conducted in compliance with Swiss Ordinance [RS 813.112.1] Good Laboratory Practice (GLP) standards, which are compatible with USEPA GLP standards (pp. 1C, 3 of MRID 49256126). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 1B-1C, 2-4). A statement of the authenticity of the study report was included as part of the Quality Assurance Statement (p. 4). |
| | ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49256127). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statement were provided (pp. 1B-1C, 2-4). An Authenticity Certification statement was not provided. |
| **Classification:** | This analytical method is classified as "**supplement**". No performance data (ECM or ILV) at 10x LOQ were provided. For the ECM, sufficient performance data at the LOQ were not provided for three of the four soil matrices. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. |
| **PC Code:** | 028828 |
| **Reviewer:** | Faruque Khan                                      **Signature:** |
| | Senior Fate Scientist                                 **Date:** December 5, 2014 |

**Executive Summary**

This analytical method, RCC Study #B18843, is designed for the quantitative determination of pyriofenone (IKF-309) in soil using HPLC/MS/MS. The method is quantitative for pyriofenone at the stated LOQ of 0.001 mg/kg. The LOQ is several orders of magnitude less than the lowest toxicological level of concern in soil. The independent laboratory validated the method for analysis of pyriofenone in sandy loam soil after one trial. No major modifications were made by the independent laboratory. The sponsor failed to provide a soil matrix of the most difficult analytical sample condition for use in the ILV. No performance data (ECM or ILV) at 10x LOQ were provided. For the ECM, sufficient performance data at the LOQ were not provided for three of the four soil matrices.

**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) | 49256126 | 49256127 | | Soil | 06/07/2007 | ISK Biosciences Corporation | HPLC/MS/MS | 0.001 mg/kg |

1 For the ECM, clay loam (organic carbon 1.90%, United Kingdom), silt loam (organic carbon 1.66%, France), loam (organic carbon 1.06%, Italy), and sand (organic carbon 0.59%, Germany) soils were used. For the ILV, a sandy loam soil (organic carbon 1.45%, North Dakota) was used.

**I. Principle of the Method**

Soil (10.0 g dry wt.) was extracted once with acetonitrile:water:hydrochloric acid (800:200:5, v:v:v; final extract volume 40 mL, volume added dependent on soil wet wt.) by shaking (laboratory shaker, *ca.* 150 rpm) for *ca.* 30 minutes (pp. 23-24 of MRID 49256126). Soil and extract were separated by centrifugation (3,000 rpm, 10 minutes). An aliquot (4 mL, equivalent to 1.0 g dry wt. soil) of the extract was diluted with ELGA purified water (20 mL), then loaded under vacuum onto a Waters Oasis HLB VAC (60 mg) solid phase extraction (SPE) cartridge pre-conditioned with methanol and ELGA water. The loaded cartridge was dried under vacuum for a few seconds. Residues were eluted with acetonitrile (5 mL). The eluate was brought to volume (10 mL) with ELGA water, mixed, and an aliquot filtered (0.45 μm PTFE) for LC/MS/MS analysis. Samples fortified at 300x LOQ were diluted 1:19 with acetonitrile:ELGA water (1:1, v:v) for analysis.

Samples were analyzed for pyriofenone (IKF-309) by HPLC (GL Sciences Inertsil ODS-3, 2.1 mm x 50 mm, 3 μm column) using a mobile phase of (A) water:acetonitrile:formic acid (95:5:0.1, v:v:v) and (B) acetonitrile:water:formic acid (95:5:0.1, v:v:v) [percent A:B (v:v) at 0.0 min. 50:50, 2.0-2.5 min. 0:100, 2.6-4.0 min. 50:50] with MS/MS-ESI (AB/MDS Sciex API 4000 triple quadrupole MS, electrospray ionization, positive ion mode) detection and multiple reaction monitoring (MRM; pp. 24-25 of MRID 49256126). Injection volume was 10 μL. Pyriofenone was identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: $m/z$ 366.0→184.3 (Q) and $m/z$ 366.0→209.3 (C). Pyriofenone retention time was *ca.* 2.3 minutes.

The ILV performed the method as written with minor method modifications (pp. 12-14, 16 of MRID 49256127). The syringe filtration (0.45 µm PTFE) step was removed from the method. Pyriofenone retention time was 3.9 minutes, due to a differing HPLC system void volume, therefore the HPLC run time was extended from 4.0 minutes to 4.5 minutes.

The LOQ for pyriofenone in soil was the same in the ECM and ILV at 0.001 mg/kg (ppm, p. 27 of MRID 49256126; p. 6 of MRID 49256127). In the ECM, the LOD was set at 0.05 ng/mL (equivalent to 0.0005 mg/kg). Although not specified, the ILV appeared to have also set the LOD at 0.05 ng/mL (Appendix B, pp. 27-28, 38-39 of MRID 49256127).

## II. Recovery Findings

ECM (MRID 49256126): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of pyriofenone (IKF-309) at fortification levels of 0.001 mg/kg (LOQ) and 0.300 mg/kg (300x LOQ) in clay loam soil (United Kingdom, n = 5 per fortification level), silt loam soil (France, n = 3 per level), loam soil (Italy, n = 3 per level), and sand soil (Germany, n = 3 per level; pp. 12-15). Recoveries from fortification at 10x LOQ (0.01 mg/kg) were not reported. Pyriofenone was identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The soil matrices were characterized (pp. 18-19).

ILV (MRID 49256127): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of pyriofenone in sandy loam soil (North Dakota) at fortification levels of 0.001 mg/kg (LOQ) and 0.1 mg/kg (100x LOQ; pp. 16-17). Recoveries from fortification at 10x LOQ (0.01 mg/kg) were not reported. 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 sandy loam soil at both fortification levels after one trial, with minor method modifications (p. 16). The soil matrix was characterized (p. 11).

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

| Matrix[1] | Fortification Level (mg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| Clay loam soil (OC 1.90%, United Kingdom) | Quantitation (primary) ion | | | | | |
| | 0.001 (LOQ) | 5 | 93-105 | 99 | 5 | 5 |
| | 0.300 | 5 | 102-110 | 106 | 4 | 4 |
| | Confirmation ion | | | | | |
| | 0.001 (LOQ) | 5 | 82-104 | 94 | 9 | 10 |
| | 0.300 | 5 | 102-109 | 106 | 3 | 3 |
| Silt loam soil (OC 1.66%, France) | Quantitation (primary) ion | | | | | |
| | 0.001 (LOQ) | 3 | 96-98 | 97 | 1 | 1 |
| | 0.300 | 3 | 76-109 | 95 | 17 | 18 |
| | Confirmation ion | | | | | |
| | 0.001 (LOQ) | 3 | 97-109 | 102 | 6 | 6 |
| | 0.300 | 3 | 75-105 | 92 | 15 | 17 |
| Loam soil (OC 1.06%, Italy) | Quantitation (primary) ion | | | | | |
| | 0.001 (LOQ) | 3 | 96-103 | 100 | 4 | 4 |
| | 0.300 | 3 | 89-99 | 95 | 5 | 5 |
| | Confirmation ion | | | | | |
| | 0.001 (LOQ) | 3 | 91-104 | 98 | 7 | 7 |
| | 0.300 | 3 | 90-101 | 96 | 6 | 6 |
| Sand soil (OC 0.59%, Germany) | Quantitation (primary) ion | | | | | |
| | 0.001 (LOQ) | 3 | 87-98 | 94 | 6 | 7 |
| | 0.300 | 3 | 97-101 | 100 | 2 | 2 |
| | Confirmation ion | | | | | |
| | 0.001 (LOQ) | 3 | 75-92 | 86 | 10 | 11 |
| | 0.300 | 3 | 97-103 | 99 | 3 | 3 |

Data (uncorrected recovery results) were obtained from Tables 1-16, pp. 35-42 of MRID 49256126 and DER Attachment 2 (standard deviations).

1 Matrix characterizations were provided (pp. 18-19 of MRID 49256126). Soil classifications are USDA. OC = organic carbon.

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

| Matrix[1] | Fortification Level (mg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| Sandy loam soil (OC 1.45%[2], North Dakota) | Quantitation (primary) ion | | | | | |
| | 0.001 (LOQ) | 5 | 93.2-95.1 | 94.3 | 0.8 | 0.8 |
| | 0.1 | 5 | 76.2-95.9 | 87.8 | 7.8 | 8.9 |
| | Confirmation ion | | | | | |
| | 0.001 (LOQ) | 5 | 82.6-92.1 | 88.0 | 4.6 | 5.2 |
| | 0.1 | 5 | 76.2-95.9 | 86.8 | 7.3 | 8.4 |

Data (uncorrected recovery results) were obtained from pp. 16-17 of MRID 49256127 and DER Attachment 2 (standard deviations).

1 Matrix characterization was provided (p. 11 of MRID 49256127). Soil classification is USDA.

2 Calculated as organic carbon (%) = organic matter (%) ÷ 1.72.

## III. Method Characteristics

In the ECM and ILV, the LOQ for pyriofenone in soil was 0.001 mg/kg (p. 27 of MRID 49256126; p. 6 of MRID 49256127). The ECM defined the LOQ as the lowest fortification level with mean recoveries of 70-110% and RSD ≤20%, and matrix control samples ≤30%. In the ECM, the LOD was estimated at 0.0005 mg/kg (0.5 µg/kg) from the lowest calibration standard of 0.05 ng/mL, with no justification (p. 27 of MRID 49256126). Although not specified, the ILV appeared to have also set the LOD at 0.05 ng/mL (Appendix B, pp. 27-28, 38-39 of MRID 49256127).

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

| | | Pyriofenone |
|---|---|---|
| Limit of Quantitation (LOQ) | | 0.001 mg/kg |
| Limit of Detection (LOD)[1] | | 0.05 ng/mL (equivalent to 0.0005 mg/kg) |
| Linearity (1/y weighting, calibration curve $r^2$ and concentration range)[2] | ECM: | Q ion: $r^2$ = 0.9982-0.9995<br>C ion: $r^2$ = 0.9981-0.9991 |
| | ILV: | Q ion: $r^2$ = 0.9986<br>C ion: $r^2$ = 0.9994 |
| | Range: | 0.05-5.0 ng/mL |
| Repeatable | | Yes |
| Reproducible | | Yes, however, the sponsor failed to provide a soil matrix of the most difficult analytical sample condition for use in the ILV. |
| Specific | | Yes |

Data were obtained from pp. 13, 27 of MRID 49256126; p. 6 of MRID 49256127.

1 LOD was not specified in ILV, but appeared to have been set at 0.05 ng/mL (Appendix B, pp. 27-28, 38-39 of MRID 49256127).

2 Linearity of the provided ECM and ILV calibration curves was verified by the reviewer (DER Attachment 2). ILV $r^2$ values are reviewer-generated from reported r values of 0.9993 (Q ion) and 0.9997 (C ion; Appendix B, pp. 27, 38 of MRID 9256127).

## IV. Method Deficiencies and Reviewer's Comments

1.    The sponsor failed to provide a soil matrix of the most difficult analytical sample condition for use in the ILV. The soil matrix was provided by the sponsor with the justification that a high organic matter soil constituted a more difficult matrix for analysis (Appendix E, p. 60 of MRID 49256127). However, the organic carbon content of the North Dakota sandy loam soil was 1.45% (calculated as 2.5% organic matter ÷ 1.72), as compared to the 1.90% organic carbon content of the clay loam (United Kingdom) soil, the soil with the highest organic carbon content in the ECM validation (pp. 18-19 of MRID 49256126; p. 11 of MRID 49256127).

2.    No performance data (ECM or ILV) at 10x LOQ were provided.

3.    For the ECM, sufficient performance data (n = 3) at both fortification levels, LOQ and 300x LOQ, were not provided for the silt loam (France), loam (Italy), and sand (Germany) soils (pp. 12-13 of MRID 49256126).

4.    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 for pyriofenone in soil

was the same in the ECM and ILV at 0.001 mg/kg (p. 27 of MRID 49256126; p. 6 of MRID 49256127). The ECM defined the LOQ as the lowest fortification level with mean recoveries of 70-110% and RSD ≤20%, and matrix control samples ≤30%. In the ECM, the LOD was estimated at 0.0005 mg/kg (0.5 µg/kg) from the lowest calibration standard of 0.05 ng/mL, with no justification (p. 27 of MRID 49256126). The LOD was not specified in the ILV but appeared to have also set at 0.05 ng/mL (Appendix B, pp. 27-28, 38-39 of MRID 49256127).

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

5.      For the ILV, chromatograms of reagent blank samples were not provided. In matrix blank control samples, interferences with peak areas were <50% at the LOD (Appendix B, pp. 28, 35, 39, 46 of MRID 49256127).

6.      For the ILV, the syringe filtration (0.45 µm PTFE) step was removed from the method due to pyriofenone losses [15-33% for 0.001 mg/kg fortified samples and 12-22% for 0.1 mg/kg fortified samples using quantitation ion (*m/z* 184.3) analysis, p. 17 of MRID 49256127]. The sponsor approved the method modification (Appendix D, p. 52 of MRID 49256127). Removal of the filtration step may only be necessary when procedural recoveries are not quantitative.

7.      It was reported for the ILV that analysis of a sample set (reagent blank, two matrix control samples and ten validation samples) required a total of 12 hours; one-working day (8 hours) for sample preparation, plus *ca*. 4 hours of LC/MS/MS analysis of samples with calibration standard injections (p. 17 of MRID 49256127).


## 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

**SMILES String:** Not found
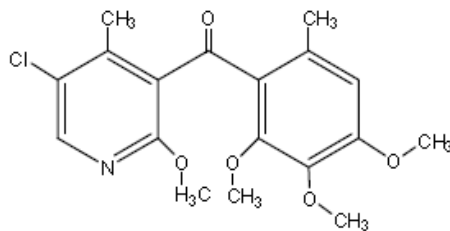


### IN-D5803

**IUPAC Name:**

**CAS Name:**

**CAS Number:**

**SMILES String:**
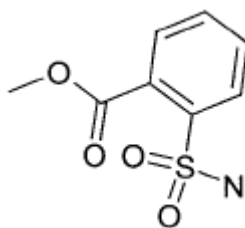
# Attachment 2: Calculations

Chemical: Pyriofenone
PC: 028828
MRIDs: 49256126 (ECM) & 49256127 (ILV)
Guideline: 850.6100
ECM Validation for Determination of Pyriofenone (IKF-309) in Soil

**Quantitation m/z 366.0 > 184.3**

| Fortified (mg a.i./kg) | Clay Loam (OC 1.90%, United Kingdom) | | | | | | | Silt Loam (OC 1.66%, France) | | | | | | | Loam (OC 1.06%, Italy) | | | | | | | Sand (OC 0.59%, Germany) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = |
| 0.001 LOQ | 105 | | | | | | | 98 | | | | | | | 96 | | | | | | | 87 | | | | | | |
| | 103 | | | | | | | 97 | | | | | | | 103 | | | | | | | 98 | | | | | | |
| | 98 | | | | | | | 96 | | | | | | | 102 | | | | | | | 98 | | | | | | |
| | 93 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 94 | 99 | 5 | 5 | 105 | 93 | 5 | | 97 | 1 | 1 | 98 | 96 | 3 | | 100 | 4 | 4 | 103 | 96 | 3 | | 94 | 6 | 7 | 98 | 87 | 3 |
| 0.300 | 103 | | | | | | | 109 | | | | | | | 89 | | | | | | | 101 | | | | | | |
| | 110 | | | | | | | 76 | | | | | | | 99 | | | | | | | 97 | | | | | | |
| | 110 | | | | | | | 101 | | | | | | | 96 | | | | | | | 101 | | | | | | |
| | 105 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 102 | 106 | 4 | 4 | 110 | 102 | 5 | | 95 | 17 | 18 | 109 | 76 | 3 | | 95 | 5 | 5 | 99 | 89 | 3 | | 100 | 2 | 2 | 101 | 97 | 3 |

**Confirmation m/z 366.0 > 209.3**

| Fortified (mg a.i./kg) | Clay Loam (OC 1.90%, United Kingdom) | | | | | | | Silt Loam (OC 1.66%, France) | | | | | | | Loam (OC 1.06%, Italy) | | | | | | | Sand (OC 0.59%, Germany) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = | Recovery (%) | Mean (%) | SD¹ (%) | RSD² (%) | Max | Min | n = |
| 0.001 LOQ | 102 | | | | | | | 97 | | | | | | | 91 | | | | | | | 75 | | | | | | |
| | 104 | | | | | | | 109 | | | | | | | 99 | | | | | | | 92 | | | | | | |
| | 91 | | | | | | | 100 | | | | | | | 104 | | | | | | | 91 | | | | | | |
| | 89 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 82 | 94 | 9 | 10 | 104 | 82 | 5 | | 102 | 6 | 6 | 109 | 97 | 3 | | 98 | 7 | 7 | 104 | 91 | 3 | | 86 | 10 | 11 | 92 | 75 | 3 |
| 0.300 | 108 | | | | | | | 105 | | | | | | | 90 | | | | | | | 98 | | | | | | |
| | 105 | | | | | | | 75 | | | | | | | 101 | | | | | | | 97 | | | | | | |
| | 109 | | | | | | | 96 | | | | | | | 96 | | | | | | | 103 | | | | | | |
| | 106 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 102 | 106 | 3 | 3 | 109 | 102 | 5 | | 92 | 15 | 17 | 105 | 75 | 3 | | 96 | 6 | 6 | 101 | 90 | 3 | | 99 | 3 | 3 | 103 | 97 | 3 |

Results from Table 1, p. 35; Table 3, p. 36; Table 5, p. 37; Table 7, p. 38; Table 9, p. 39; Table 11, p. 40; Table 13, p. 41; Table 15, p. 42 of MRID 49256126. Soil classifications are USDA (pp. 18-19 of MRID 49256126).

Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
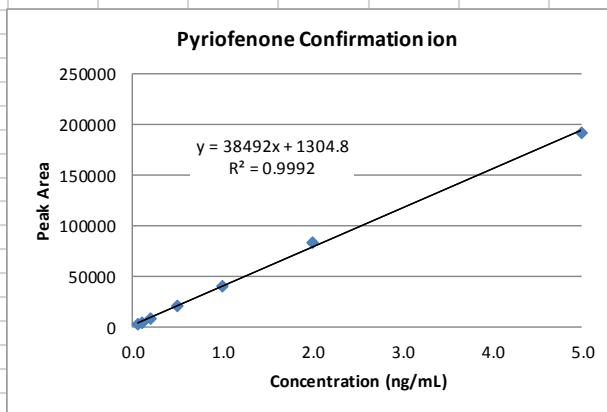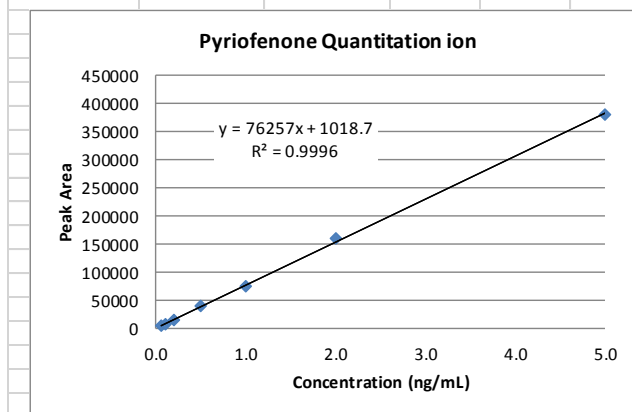
1 SD = Standard Deviation; determined using the "unbiased" or "n-1" method.

2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

Chemical: Pyriofenone
PC: 028828
MRIDs: 49256126 (ECM) & 49256127 (ILV)
Guideline: 850.6100
ECM Calibration Curves

| Concentration (ng/mL) | Pyriofenone (IKF-309) | |
| --- | --- | --- |
| | Quantitation | Confirmation |
| | Peak Area Counts | Peak Area Counts |
| 0.05 | 3795 | 2146 |
| 0.10 | 7896 | 4138 |
| 0.20 | 15457 | 8519 |
| 0.50 | 39888 | 20362 |
| 1.00 | 75451 | 40027 |
| 2.00 | 159090 | 82487 |
| 5.00 | 380432 | 192109 |

Results (Peak Area Counts) from Tables 17-18, pp. 43-44 of MRID 49256126.



Pyriofenone Quantitation ion

$y = 76257x + 1018.7$
$R^2 = 0.9996$



Pyriofenone Confirmation ion

$y = 38492x + 1304.8$
$R^2 = 0.9992$

Chemical: Pyriofenone

PC: 028828

MRIDs: 49256126 (ECM) & 49256127 (ILV)

Guideline: 850.6100

ILV for Determination of Pyriofenone (IKF-309) in Soil

| Fortified (mg a.i./kg) | Recovery (%) | Mean (%) | SD[1] (%) | RSD[2] (%) | Max | Min | n = |
|---|---|---|---|---|---|---|---|
| Sandy Loam (OC 1.45%, North Dakota) | | | | | | | |
| Quantitation m/z 366.0 > 184.3 | | | | | | | |
| 0.001 | 94.0 | | | | | | |
| LOQ | 94.9 | | | | | | |
| | 93.2 | | | | | | |
| | 95.1 | | | | | | |
| | 94.2 | 94.3 | 0.8 | 0.8 | 95.1 | 93.2 | 5 |
| 0.1 | 92.6 | | | | | | |
| | 95.9 | | | | | | |
| | 90.4 | | | | | | |
| | 76.2 | | | | | | |
| | 83.9 | 87.8 | 7.8 | 8.9 | 95.9 | 76.2 | 5 |
| Confirmation m/z 366.0 > 209.3 | | | | | | | |
| 0.001 | 82.6 | | | | | | |
| LOQ | 90.4 | | | | | | |
| | 91.4 | | | | | | |
| | 92.1 | | | | | | |
| | 83.5 | 88.0 | 4.6 | 5.2 | 92.1 | 82.6 | 5 |
| 0.1 | 89.7 | | | | | | |
| | 95.9 | | | | | | |
| | 88.1 | | | | | | |
| | 76.2 | | | | | | |
| | 83.9 | 86.8 | 7.3 | 8.4 | 95.9 | 76.2 | 5 |

Results from pp. 16-17 of MRID 49256127. Soil classification is USDA (p. 11 of MRID 49256127).

OC = organic carbon calculated as organic carbon (%) = organic matter (%) ÷ 1.72 (p. 11 of MRID 49256127).

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: 49256126 (ECM) & 49256127 (ILV)
Guideline: 850.6100
ECM Calibration Curves

| Concentration (ng/mL) | Pyriofenone (IKF-309) | |
| --- | --- | --- |
| | Quantitation | Confirmation |
| | Peak Area Counts | Peak Area Counts |
| 0.05 | 2040 | 1420 |
| 0.10 | 3550 | 2640 |
| 0.20 | 7970 | 5500 |
| 0.50 | 19300 | 13700 |
| 1.00 | 44900 | 30300 |
| 2.00 | 88500 | 60200 |
| 5.00 | 215000 | 147000 |

Results (Peak Area Counts) from Appendix B, pp. 28-34, 39-45 of MRID 49256127.



Pyriofenone Quantitation ion

$y = 43246x - 209.18$
$R^2 = 0.9996$



Pyriofenone Confirmation ion

$y = 29524x - 75.394$
$R^2 = 0.9998$