KIH-485, M-1 and M-3 Water Method as Described in "Aquatic Field Dissipation of Residues Following Application of KIH-485 WG85 to Water," Janine E. Marin, Ph.D., PTRL West Study 1662W

This method is used for the determination of KIH-485, M-1 and M-3 in water. This method has a demonstrated limit of quantitation of 0.005 mg/L.

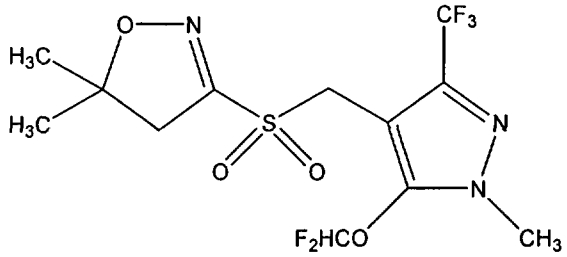
MATERIALS AND METHODS

Reference Substances

The KIH-485 (1472W-001A) reference substance was provided by Kumiai Chemical, Inc. with a purity of 99.9%, with an expiration date of September 29, 2007 (99.74% by Certificate of Analysis dated July 20, 2007, with expiration date of July 19, 2009). The KIH-485 M-1 metabolite reference substance (1472W-002) was provided with a purity of 97.8% and expiration date of December 19, 2007 (97.54% by Certificate of Analysis dated November 30, 2007, with expiration date of November 29, 2009). The KIH-485 M-3 metabolite reference substance (1472W-003) was provided with a purity of 99.6% and expiration date of December 18, 2007 (99.6% by Certificate of Analysis dated November 30, 2007, with expiration date of November 30, 2009). Stock solutions of each reference substance were prepared at 1 mg/mL in acetone. Mixed dilutions of KIH-485, M-1 and M-3 were prepared as described below. The reference substance solution were taken to be stable if stored frozen for approximately 6 months, based on the comparison of LCMS chromatograms of the analyses.

Statement of Analytical Reference Substances

The following standards were utilized for analysis throughout the study (see Appendix B for Certificates of Analysis):



KIH-485

Compound: **KIH-485**

Chemical Name: 3-[(5-difluoromethoxy-1-methyl-3-trifluoromethylpyrazol-4-yl)-methylsulfonyl]-4,5-dihydro-5,5-dimethylisoxazole

CAS No.: 447399-55-5

Purity: 99.9% (99.74%)

Molecular Weight: 391.3 g/mole

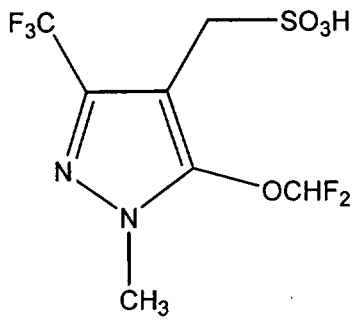
Lot Number: LP001

Supplier: Kumiai Chemical Industry Co., Ltd.

Date Received: January 30, 2006 (December 18, 2007)

Expiration Date: September 29, 2007 (July 19, 2009)

Storage Conditions: <30°C



Compound: **KIH-485 M-1**

Chemical Name: (5-difluoromethoxy-1-methyl-3-trifluoromethyl-1*H*-pyrazol-4-yl) methanesulfonic acid

CAS No.: NA

Purity: 97.8% (97.54%)

Molecular Weight: 310.20 g/mole

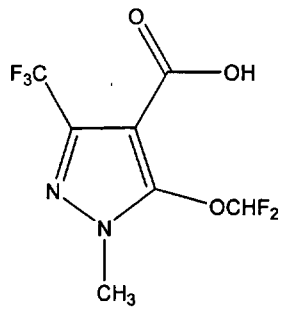
Lot Number: 2

Supplier: Kumiai Chemical Industry Co., Ltd.

Date Received: February 15, 2007 (December 18, 2007)

Expiration Date: December 19, 2007 (November 29, 2009)

Storage Conditions: Approximately 4°C



Compound: **KIH-485 M-3**

Chemical Name: 5-difluoromethoxy-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid

CAS No.: NA

Purity: 99.6% (99.6%)

Molecular Weight: 260.12 g/mole

Lot Number: 4

Supplier: Kumiai Chemical Industry Co., Ltd.

Date Received: February 18, 2007 (December 15, 2007)

Expiration Date: December 18, 2007 (November 29, 2009)

Storage Conditions: Approximately 4°C

Solvents (HPLC grade or better)

Acetone

Acetonitrile

Methanol

Water

Glassware and Miscellaneous Equipment

Balance

Centrifuge, Eppendorf 5415C

Graduated cylinder, various sizes

Microfilterfuge tubes, Rainin (0.45 µm, Catalog no. 7016-022)

Pasteur pipettes

Syringes, microliter, various sizes

Vials, amber (2 mL capacity) with Teflon®-lined crimp cap, Chromacol, Inc., Trumbull, CT

Volumetric flask, various sizes

Volumetric pipette, various sizes

ANALYTICAL PROCEDURES

Preparation of Standards

Stock standard solutions of KIH-485 (lot # LP001), M-3 (lot # 4) and M-1 (lot # 2) were prepared at 1 mg/mL in acetonitrile. The stock standards were stored frozen (< 0°C) when not in use.

Preparation of Fortification Standards

KIH-485 plus M-3 and M-1 fortification standards were prepared by dilution of a 1 mg/mL stock standard with acetonitrile (0.250 mL diluted to 25 mL) to yield a 10.0 µg/mL fortification standard. An additional fortification solution was prepared by dilution with acetonitrile to yield a mixed fortification solution at 1.0 µg/mL. Dilutions were prepared using Hamilton syringes, volumetric flasks and volumetric pipettes. The fortification standards were stored at <0°C when not in use.

Fortification Procedure

Fortification of untreated water with KIH-485, M-3 and M-1 was performed to analyze method percent recoveries for method validation and for sample set analysis. A portion (5 mL of water) was fortified as shown below:

Fortification Level (mg/kg)	Mixed Stock Standard
0.005	25 µL of 1.0 µg/mL mixed fort stock
0.01	50 µL of 1.0 µg/mL mixed fort stock
0.05	25 µL of 10 µg/mL mixed fort stock

Preparation of Linearity Standards

Dilutions of the 10 µg/mL fortification standards were used to prepare the following linearity standards. All KIH-485 and M-3 dilutions were prepared with acetone. Standards were prepared using volumetric flasks and Hamilton syringes. KIH-485 and M-3 calibrants were stored frozen when not in use.

Concentration of KIH-485, M-3 and M-1 (ng/mL, each)	**Mixed Standard Solution Used**	**Volume of Mixed Standard added**	**Final Volume (mL)**
0.5	10.0 µg/mL	12.5 µL	25
1.0	10.0 µg/mL	25 µL	25
5.0	10.0 µg/mL	125 µL	25
10.0	10.0 µg/mL	250 µL	25
25	1.0 µg/mL	625 µL	25
50	1.0 µg/mL	125 µL	25
100	1.0 µg/mL	250 µL	25

PREPARATION OF SAMPLES

KIH-485, M-1 & M-3 Water Method

1. Partially fill a 5 mL volumetric flask with control water.
2. Spike samples, as needed. Dilute to the mark with control water.
3. Microfilterfuge field and spiked control samples, as necessary, prior to LC-MS/MS analysis.

LC/MS/MS Analysis of KIH-485, M-3, and M-1

SCIEX 3000 or 4000 Components (HPLC/Turbo Ion Spray Mode):

LC Pump	Agilent 1100 Series Binary Pump, Model G1312A
Autosampler	Agilent 1100 Series Autosampler, Model G1313A
Controller	Agilent 1100 Series Handheld Control Module G1323B
Divert Valve	Valco Switching Valve

Column: Michrom C18, 15 cm x 3.2 mm x 5µ with
Upchurch pre-column (1 cm x 4.6mm ODS)

METHOD A for KIH-485 and M-3 in water:

Solvent System and Gradient Program:

Solvent A = Water (0.05% formic acid)

Solvent B = Acetonitrile (0.05% formic acid)

Flow Rate: 0.25 mL/minute

Solvent Program:

Minutes	Solvent A	Solvent B
0	95	5
20.0	0	100
25.0	0	100
27.0	95	5
35.0	95	5

Period 1 settings: Experiment 1:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
392	229	500 (or 100)
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
392	179	500 (or 100)

Representative Mass Spectrometer Settings

	Period 1 API 3000	Period 1 API 4000
Scan Type:	MRM	MRM
Polarity:	Positive	Positive
Ion Source:	Turbo Spray	Turbo Spray
NEB:	11.0	---

CUR:	13.0	35.0
CAD:	6.0	10.0
GS1:	---	40.0
GS2:	---	60.0
IS:	5000.0	3500.0
TEMP:	0.0	450.0
DP:	30.0	70.0
FP:	150.0	---
EP:	6.0	10.0

Period 1 settings: Experiment 2:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
259	215	100
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
259	165	100
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
309	259	100
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
309	195	100

Representative Mass Spectrometer Settings

	Period 1 API 3000	Period 1 API 4000
Scan Type:	MRM	MRM
Polarity:	Negative	Negative
Ion Source:	Turbo Spray	Turbo Spray
NEB:	11.0	---
CUR:	13.0	35.0
CAD:	8.0	10.0
GS1:	---	40.0
GS2:	---	60.0
IS:	-4000.0	-3500.0
TEMP:	450.0	450.0
DP:	-30.0	-30.0

FP:	-150.0	---
EP:	-6.0	-10.0

Retention Time: KIH-485 at ~17 minutes for m/z 229 + m/z 179, M-3 at ~14 minutes for m/z 215+m/z 165

METHOD B for KIH-485, M-3 and M-1 in water:

Solvent System and Gradient Program:

Solvent A = Water (0.05% formic acid)

Solvent B = Methanol (0.05% formic acid)

Solvent Program:

Minutes	mL/min	Solvent A	Solvent B
0	230	90	10
5.0	230	90	10
20.0	230	0	100
24.0	350	0	100
25.0	230	90	10
31.0	230	90	10

Period 1 settings: Experiment 1:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
392	229	100
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
392	179	100

Representative Mass Spectrometer Settings

	Period 1 API 3000
Scan Type:	MRM
Polarity:	Positive
Ion Source:	Turbo Spray
NEB:	11.0
CUR:	13.0
CAD:	6.0
GS1:	---
GS2:	---
IS:	5000.0

TEMP:	0.0
DP:	30.0
FP:	150.0
EP:	6.0

Period 1 settings: Experiment 2:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
259	215	100
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
259	165	100
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
309	259	100
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
309	195	100

Representative Mass Spectrometer Settings

	Period 1 API 3000
Scan Type:	MRM
Polarity:	Negative
Ion Source:	Turbo Spray
NEB:	11.0
CUR:	13.0
CAD:	8.0
GS1:	---
GS2:	---
IS:	-4000.0
TEMP:	450.0
DP:	-30.0
FP:	-150.0
EP:	-6.0

Retention Time: KIH-485 at ~20 minutes for m/z 229 + m/z 179, M-3 at ~19 minutes for m/z 215 + m/z 165 and M-1 at ~18 minutes for m/z 259 + m/z 195.

Separation of KIH-485, M-1 and M-3 was achieved by high performance liquid chromatography. The analytes were identified by the coincidence of their retention times with that of the respective reference standards.

Methods of Calculation:

Preparation of Stock Standards

$$\text{Volume of solvent (mL)} = \frac{(W) \times (P)}{(FC)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (mg/mL)

The KIH-485 quantitation was conducted by peak area relative to and external calibration curve. A calibrant peak area (y) relative to the concentration of the calibrant in ng/mL (x) yielded a linearity curve, where y = mx + b was plotted. Similar calibration curves were generated for M-1 and M-3.

The residue of KIH-485 in water was calculated as follows:

mg/L KIH-485 (ppm) = µg/mL KIH-485 x Dil. Factor x 0.001 mg/µg

$$\% \text{ Recovery} = \frac{\text{KIH - 485 Residue Detected (mg/L) - KIH - 485 Residue in control}}{\text{KIH - 485 Fortification Level (mg/L)}} \times 100$$

Similar calculations were conducted for M-1 and M-3, using the corresponding calibration curves.

To demonstrate validity of the analytical method for acceptable recovery (70-120%) of the KIH-485, M-1 and M-3 from water, method validation sets were conducted with replicates of fortified control water samples at three different fortification levels (0.005

mg/L, 0.01 mg/L and 0.10 mg/kg). Residues of all three analytes in treated samples were calculated as shown above, with no control residues subtracted.

An example calculation for the KIH-485 residue in water (Fort 1 at 0.005 mg/L in CA water method validation) is shown below:

Linear regression analysis of the KIH-485 standards gave a curve with the equation x = (y – 9,994) ÷ 23,308,026 (r^2 = 0.9995). The µg/mL KIH-485 injected determined by this curve was:

µg/mL KIH-485 = [(110,930 – 9,994) ÷ 23,308,026] = 0.0043 µg/mL = 0.0043 mg/L

$$\text{Percent KIH-485 Recovery} = \frac{0.0043\ \text{mg/L} - 0.0000\ \text{mg/L}}{0.005\ \text{mg/L}} \times 100 = 86.6\%$$

Similar calculations were carried out for M-3 and M-1 in water.