

## 1 Summary

### Study Objective

The objective of the study was to independently validate the analytical method for the determination of IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990 (sum of diastereomers), and pyrazole carboxamide in/on soil in accordance to the guidance document OSCPP 860.1340. The limit of quantification is 0.005 mg/kg (dry weight) for each analyte.

### Analytical Procedure

In brief, the soil samples were extracted three times with acetone/water (90/10, v/v), acetone/water (50/50, v/v) and acetone/0.5 N HCl (50/50, v/v) using a sonicator and/or a reciprocal shaker. After centrifugation and evaporating off the acetone, the remaining water part was diluted with methanol for HPLC-MS/MS analysis.

### Selectivity

Quantification was performed by use of HPLC-MS/MS detection. Two (2) mass transitions for each analyte were evaluated in order to demonstrate that the method achieves a high level of specificity. No significant interference above 20 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each soil type, so that a high level of selectivity was demonstrated.

### Matrix Effects

Matrix effects on the detection of all analytes in final extracts of a sandy loam soil from Nebraska, USA, and a silt loam soil from New York, USA, were evaluated.

Matrix effects on the detection of IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990, and pyrazole carboxamide in final extracts of both types of soil were found to be insignificant ( $< \pm 20\%$ ), except 3-hydroxy-IR9792/F9990 in silt loam soil from New York. However, matrix-matched standards were used for quantification.

### Linearity

For IR9792/F9990, 3-hydroxy-IR9792/F9990, and pyrazole carboxamide, the linearity of the detector response was demonstrated by determination of matrix-matched calibration standards at six (6) concentration levels ranging from 0.10 ng/mL to 10 ng/mL for both soil types. This range corresponds to 0.002 mg/kg to 0.20 mg/kg and thus covers the range from no more than 40 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a final sample extract.

For 1-carboxy-IR9792/F9990 (diastereomer a), the linearity of the detector response was demonstrated by determination of matrix-matched calibration standards at six (6) concentration levels ranging from 0.0632 ng/mL to 6.32 ng/mL for both soil types. This range corresponds to 0.0013 mg/kg to 0.13 mg/kg and thus covers the range from no more than 40 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a final sample extract.

For 1-carboxy-IR9792/F9990 (diastereomer b), the linearity of the detector response was demonstrated by determination of matrix-matched calibration standards at six (6) concentration levels ranging from 0.0368 ng/mL to 3.68 ng/mL for both soil types. This range corresponds to 0.0007 mg/kg to 0.07 mg/kg and thus covers the range from no more than 40 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a final sample extract.

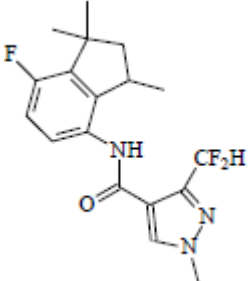
The calibration curves obtained for both mass transitions were linear with correlation coefficients ( $r \geq 0.995$ ). Linear regression was performed with 1/x-weighting.

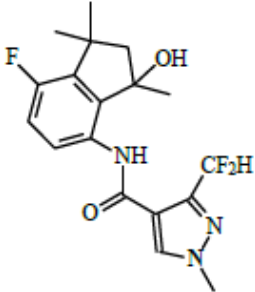
## 2 Study Objective

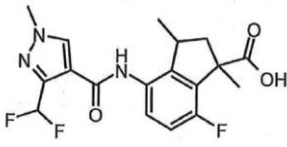
The objective of the study was to independently validate the analytical method for the determination of IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990 (sum of diastereomers), and pyrazole carboxamide in/on soil in accordance to the guidance document OSCPP 860.1340. The limit of quantification is 0.005 mg/kg for each analyte.

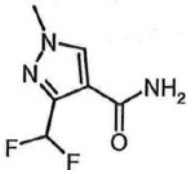
## 3 Materials and Methods

### 3.1 Test / Reference Item(s)

Common name	F9990	Synonym	IR9792/F9990
Chemical name (IUPAC)	3-(difluoromethyl)-N-[(3RS)-7-fluoro-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methylpyrazole-4-carboxamide		
Chemical structure		CAS number	1383809-87-7
		Empirical formula	C <sub>18</sub> H <sub>20</sub> F <sub>3</sub> N <sub>3</sub> O
		Molecular weight	351.4 g/mol
Supplier	FMC Corporation	Purity analysed	98.6 % (w/w)
EAS test item code	PA-0059	Batch / Lot number	PL13-0195
Date of certificate / analysis	27 Sep 2016	Expiry date	Jul 2018
		Storage conditions at testing facility	ambient, dark, dry

Reference Item 2			
Common name	3-Hydroxy-F9990	Synonym	3-Hydroxy-F9990/IR9792
Chemical name (IUPAC)	3-(difluoromethyl)-N-(7-fluoro-3-hydroxy-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide		
Chemical structure		CAS number	none
		Empirical formula	C <sub>18</sub> H <sub>20</sub> F <sub>3</sub> N <sub>3</sub> O <sub>2</sub>
		Molecular weight	367.4 g/mol
Supplier	FMC Corporation	Purity analysed	96.7 % (w/w)
EAS test item code	PA-0065	Batch / Lot number	30440/43
Date of certificate / analysis	31 Mar 2017	Expiry date	Mar 2019
		Storage conditions at testing facility	≤ - 18 °C, dark, dry

Reference Item 3			
Common name	1-Carboxy- IR9792/F9990	Synonym	F9990-1-Carboxylate
Chemical name (IUPAC)	4-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-7-fluoro-1,3-dimethylindane-1-carboxylic acid		
Chemical structure		CAS number	NA
		Empirical formula	C <sub>18</sub> H <sub>18</sub> F <sub>3</sub> N <sub>3</sub> O <sub>3</sub>
		Molecular weight	381.36 g/mol
Supplier	FMC Corporation	Purity analysed	93.7 % (w/w); Diastereomer Ratio = 1.72:1
EAS test item code	PA-0057	Batch / Lot number	55261-4-8
Date of certificate / analysis	23 Mar 2017	Expiry date	Mar 2019
		Storage conditions at testing facility	≤ - 18 °C, dark, dry

Common name	3-(Difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide	Synonym	Pyrazole carboxamide
Chemical structure		CAS number	925689-10-7
		Empirical formula	C <sub>6</sub> H <sub>7</sub> F <sub>2</sub> N <sub>3</sub> O
		Molecular weight	175.14 g/mol
Supplier	FMC Corporation	Purity analysed	99.5 % (w/w)
EAS test item code	PA-0061	Batch / Lot number	R14151
Date of certificate / analysis	23 Jun 2017	Expiry date	Apr 2019
		Storage conditions at testing facility	≤ - 18 °C, dark, dry

Specifications essential for correct identification of the reference item(s) and for use under GLP are based on the information as provided by the study sponsor (e. g. certificate(s) of analysis). They have not been verified by the test facility and might have not been generated under GLP.

Copies of the certificates of analysis are included in Appendix F.

### 3.2 Test System(s), Sample Origin, Preparation and Storage

Soil (sandy loam and silt loam)	Certified standard soil types were received on 03 Oct 2017 from SGS NAM GLP Laboratory.	The soil samples were received in a homogenized state. Additional preparation was not necessary.	The soil samples were stored at room temperature in the dark until fortification and extraction dates.
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### 3.3 Soil Characteristics

	Nebraska, USA (SGS Soil ID 14-02-04 UTC-SC-1-CTR-6)	New York, USA (SGS Soil ID 14-02-02 UTC-SC-1-CTR-6)
USDA Textural Class (hydrometer method)	Loamy sand	Silt loam
Soil depth	0-6"	0-6"
% Sand > 1.00 mm	1.0	0.0
% Sand > 500 µm & < 1.00 mm	5.9	0.5
% Sand > 250 µm & < 500 µm	39.4	1.1

	<b>Nebraska, USA</b> <b>(SGS Soil ID 14-02-04 UTC-SC-1-CTR-6)</b>	<b>New York, USA</b> <b>(SGS Soil ID 14-02-02 UTC-SC-1-CTR-6)</b>
% Sand > 180 µm & < 250 µm	22.9	0.6
% Sand > 106 µm & < 180 µm	6.4	1.5
% Sand > 45 µm & < 106 µm	5.4	25.6
Percent Sand	83	25
Percent Soil	10	60
Percent Clay	7	15
Bulk Density (disturbed) gm/cc	1.34	0.97
Cation Exchange Capacity (meq/100 g)	8.8	8.2
% Moisture at 1/3 bar	10.4	28.6
% Moisture at 15 bar	4.6	9.9
% Organic Carbon – Walkley Black	0.90	2.5
% Organic Matter – Walkley Black	1.5	4.3
pH in 1/1 soil/water ratio	6.0	5.1
Olsen Phosphorus (ppm)	40	Not performed
Total Nitrogen (Analyzed) (%)	0.09	Not performed
Soluble Salts (mmhos/cm)	0.13	Not performed
Calcium	47.4 % (831 ppm)	18.7 % (307 ppm)
Magnesium	11.7 % (123 ppm)	6.1 % (60 ppm)
Sodium	0.3 % (7 ppm)	0.5 % (9 ppm)
Potassium	5.9 % (201 ppm)	2.8 % (90 ppm)
Hydrogen	34.7 % (30 ppm)	72 % (59 ppm)

### 3.4 Method Summary

In brief, the soil samples were extracted three times with acetone/water (90/10, v/v), acetone/water (50/50, v/v) and acetone/0.5 N HCl (50/50, v/v) using a sonicator and/or a reciprocal shaker. After centrifugation and evaporation off the acetone, the remaining water part was diluted with methanol for HPLC-MS/MS analysis.

The full method descriptions, including flow charts, are given in Appendix A.

No addition or modification to the original method was done, except the optimization of instrumental parameters to archive the sufficient sensitivity and using of reciprocal shaker instead of wrist action shaker.

No communication with the method developers or others familiar with the method was necessary to carry out the analysis.

## **4 Validation Procedure, Results and Discussion**

### **4.1 Specificity and Selectivity**

HPLC-MS/MS determination was conducted by monitoring two (2) mass transitions for each analyte. Mass transitions 352→332 *m/z* for IR9792/F9990, 366→175 *m/z* for 3-hydroxy-IR9792/F9990, 382→336 *m/z* for 1-carboxy-IR9792/F9990 diastereomers, and 176→136 *m/z* for pyrazole carboxamide are proposed to be used for quantification but all mass transitions are applicable interchangeably for quantification and confirmation.

A reagent blank and at least two (2) control samples per soil type were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analytes. For all mass transitions, the samples showed no significant interference (above 20 % of LOQ) at the retention time of the analytes in any investigated soil type, therefore showing that the method is highly specific.

Blank correction was not performed.

Example chromatograms for each soil type and analyte representing control samples, the lowest and highest calibration levels, samples fortified at the LOQ and samples fortified at 10x LOQ and also showing reagent blank extracts are all included in the figures section (Appendix D).

In addition, MS/MS spectra of each analyte are also presented in Appendix B.

No addition or modification to the original method was done, except the optimization of instrumental parameters to archive the sufficient sensitivity and using of reciprocal shaker instead of wrist action shaker. No communication with the method developers or others familiar with the method was necessary to carry out the analysis and the first attempt made resulted in the validation data reported here.

## **7 Reference List**

- [1] United States Environmental Protection Agency (EPA) Residue Chemistry Test Guidelines: "OPPTS 860.1340 Residue Analytical Method", August 1996.
- [2] Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Good Laboratory Practices (GLP) Regulations: Title 40 "Protection of Environment", Part 160, Volume 24, 01-Jul-2011.
- [3] Christopher Skaggs, SGS North America, Inc.: "Method Validation of IR97792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990 (diastereomer 1), 1-carboxy-IR9792/F9990 (diastereomer 2), and pyrazole carboxamide in soil."

## Appendix A

### Analytical Method

#### I. Reagents and Materials

Information pertaining to the identity and source of reagents is summarised in Table I.

**Table I: Identification of Reagents and Materials**

<ul style="list-style-type: none"> <li>• Methanol HPLC grade (Fisher Scientific, Lot No. 171000)</li> <li>• Acetone ASC grade (Fisher Scientific, Lot No. 143956)</li> <li>• Water HPLC grade (Fisher Scientific, Lot No. 173784)</li> <li>• Hydrochloric acid solution 6N (Fisher Scientific, Lot No. 163081)</li> <li>• Ammonium acetate 98.8 % (Fisher Scientific, Lot No. 161197A)</li> <li>• Formic acid <math>\geq 98</math> % (Acros organics, Lot No. A0380211)</li> <li>• Polypropylene centrifuge tubes (50 mL)</li> <li>• Common laboratory glassware</li> <li>• Adjustable pipettes (Eppendorf: Research Plus 20-200 <math>\mu</math>L, Research 100-1000 <math>\mu</math>L, Gilson: M250 50-250 <math>\mu</math>L)</li> </ul>
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## II. Instrumentation and Apparatus

Information pertaining to the identity of instruments and apparatus is summarised in Table II.

**Table II: Identification of Instrumentation and Apparatus**

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| <ul style="list-style-type: none"><li>• Balances<br/>(Mettler AT261 DeltaRange; Mettler-Toledo PC440;)</li><li>• Sonicator<br/>(Branson Ultrasonic Cleaner, Brason Model 3510R-DTH)</li><li>• Evaporator<br/>(Turbo VapLV, Zymark)</li><li>• Reciprocal shaker<br/>(Eberbach 6010)</li><li>• Laboratory centrifuges<br/>(Allegra 6KR, Beckman Coulter; Allegra X-14R, Beckman Coulter)</li><li>• Vortex mixer<br/>(Mini Vortexer MV1, VWR)</li><li>• HPLC- MS/MS<br/>(Applied Biosystems Sciex API 4000 with Shimadzu Nexera X2 HPLC)</li></ul> |
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### III. Reagent Solutions and Mobile Phases

Description	Composition
Acetone/Water (50/50, v/v)	For example, add 500 mL of acetone and 500 mL of HPLC water into a 1000 mL glass bottle and mix well to ensure complete homogenous solution.
Acetone/(0.5 N HCl in water) (50/50, v/v)	For example, add 500 mL of acetone, 458 mL of HPLC water and 42 mL of hydrochloric acid solution 6N into a 1000 mL glass bottle and mix well to ensure complete homogenous solution.
Acetone/Water (90/10, v/v)	For example, add 1800 mL of acetone and 200 mL of HPLC water into a 2000 mL glass bottle and mix well to ensure complete homogenous solution.
Methanol/Water (80/20, v/v)	For example, add 800 mL of methanol and 200 mL of HPLC water into a 1000 mL glass bottle and mix well to ensure complete homogenous solution.
Washing Solution	Methanol/Water (50/50, v/v) For example, add 500 mL of methanol and 500 mL of HPLC water into a 1000 mL glass bottle and mix well to ensure complete homogenous solution.
HPLC mobile phase A	HPLC Water + 10 mM ammonium acetate + 0.2 % formic acid For example, weigh ≈771 mg of ammonium acetate into 1.0 L glass bottle, add 1.0 L of HPLC water and 2 mL of formic acid. Mix well to ensure complete homogenous solution.
HPLC mobile phase B	Methanol + 0.2 % formic acid For example, add 2 mL of formic acid and 1000 mL of methanol into a 1000 mL glass bottle and mix well to ensure complete homogenous solution.

## IV. Preparation of Standard Solutions

### 1) Stock Solutions

The stock solutions of IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990, and pyrazole carboxamide were prepared by dissolving a weight of the reference items in methanol with the aid of an ultrasonic bath and were stored refrigerated (2 °C to 8 °C) in a glass vial in the dark.

A summary of the preparation is presented in the following tables:

**Table III: Preparation of Stock Solution of IR9792/F9990 in Methanol**

Purity of reference item* (%)	Weighed amount of reference item (mg)	Amount of analyte corrected for purity (mg)	Final volume (mL)	Final concentration (µg/mL)	Reference of standard solution produced
98.6	10.29	10.15	10.145	1000	PA-0059-01
98.6	10.44	10.29	10.294	1000	PA-0059-02**

\* taken from the Certificate of Analysis.

\*\* The diluted stock solution was used for the instrument optimisation.

**Table IV: Preparation of Stock Solution of 3-hydroxy-IR9792/F9990 in Methanol**

Purity of reference item* (%)	Weighed amount of reference item (mg)	Amount of analyte corrected for purity (mg)	Final volume (mL)	Final concentration (µg/mL)	Reference of standard solution produced
96.7	10.69	10.34	10.337	1000	PA-0065-01
96.7	10.39	10.05	10.047	1000	PA-0065-02**

\* taken from the Certificate of Analysis.

\*\* The diluted stock solution was used for the instrument optimisation.

**Table V: Preparation of Stock Solution of 1-carboxy-IR9792/F9990 in Methanol**

Purity of reference item* (%)	Weighed amount of reference item (mg)	Amount of analyte corrected for purity (mg)	Final volume (mL)	Final concentration (µg/mL)	Reference of standard solution produced
93.7 (a+b)	10.71 (a+b)	10.3 (a+b)	10.035	632 (a)**; 368 (b)**; 1000 (a+b)	PA-0057-01
93.7 (a+b)	10.70 (a+b)	10.3 (a+b)	10.026	632 (a)**; 368 (b)**; 1000 (a+b)	PA-0057-02***

\* taken from the Certificate of Analysis.

\*\* Diastereomer Ratio =1.72/1 (a/b).

\*\*\* The diluted stock solution was used for the instrument optimisation.

**Table VI: Preparation of Stock Solution of Pyrazole Carboxamide in Methanol**

Purity of reference item* (%)	Weighed amount of reference item (mg)	Amount of analyte corrected for purity (mg)	Final volume (mL)	Final concentration (µg/mL)	Reference of standard solution produced
99.5	10.20	10.15	10.149	1000	PA-0061-01
99.5	10.34	10.29	10.288	1000	PA-0061-02**

\* taken from the Certificate of Analysis.

\*\* The diluted stock solution was used for the instrument optimisation.

## 2) Intermediate and Fortification Solutions

The stock solutions were further diluted for use as fortification solutions in the recovery process and as (intermediate) standard solutions for subsequent use as solvent calibration solutions and/or preparation of matrix-matched calibration solutions.

All solutions were stored refrigerated (2 °C to 8 °C) in a glass vial in the dark.

A summary of the typical dilutions is presented in the following table.

**Table VII: Preparation of Intermediate and Fortification Solutions in Methanol**

Reference of standard solution used	Concentration (µg/mL)	Volume taken (mL)	Final volume (mL)	Final concentration (µg/mL)	Reference of standard solution produced
PA-0059-01	1000	0.200	20	10	PA-0059-01-01
PA-0065-01	1000	0.200			
PA-0057-01	1000 (a+b)/632 (a)/368 (b)	0.200		10 (a+b)/6.32 (a)/3.68 (b)	
PA-0061-01	1000	0.200		10	
PA-0059-01-01	10 (each,a+b)/6.32 (a)/3.68 (b)	2.0	20	1.0 (each,a+b)/0.632 (a)/0.368 (b)	PA-0059-01-02
PA-0059-01-02	1.0 (each,a+b)/0.632 (a)/0.368 (b)	2.0	20	0.100 (each,a+b)/0.0632 (a)/0.0368 (b)	PA-0059-01-03

## 3) Matrix-matched Calibration Solutions

Matrix-matched calibration solutions were prepared using final sample extracts of control (untreated) samples of a respective soil type fresh on the day of analysis by several dilution of the intermediate solvent solution. A summary of the typical dilutions is presented in the following table.

**Table VIII: Preparation of Matrix-matched Calibration Solutions of IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990, and Pyrazole Carboxamide in the Final Sample Extracts of Soil**

Reference of standard solution used	Concentration (ng/mL)	Volume taken (µL)	Final volume (µL)	Final concentration (ng/mL)	Reference of standard solution produced
PA-0059-01-02	1000 (each,a+b)/632 (a)/368 (b)	100	1000	100 (each,a+b)/63.2 (a)/36.8 (b)	mStd Mix 100 ng/mL (not injected)
mStd Mix 100 ng/mL	100 (each,a+b)/63.2 (a)/36.8 (b)	100	1000	10 (each,a+b)/6.32 (a)/3.68 (b)	mStd Mix 10 ng/mL

**Table VIII: Preparation of Matrix-matched Calibration Solutions of IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990, and Pyrazole Carboxamide in the Final Sample Extracts of Soil (continued)**

Reference of standard solution used	Concentration (ng/mL)	Volume taken (µL)	Final volume (µL)	Final concentration (ng/mL)	Reference of standard solution produced
mStd Mix 100 ng/mL	100 (each,a+b)/ 63.2 (a)/36.8 (b)	50	1000	5.0 (each,a+b)/ 3.16 (a)/1.84 (b)	mStd Mix 5.0 ng/mL
mStd Mix 100 ng/mL	100 (each,a+b)/ 63.2 (a)/36.8 (b)	25	1000	2.50 (each,a+b)/ 1.58 (a)/0.920 (b)	mStd Mix 2.5 ng/mL
mStd Mix 10 ng/mL	10 (each,a+b)/ 6.32 (a)/3.68 (b)	100	1000	1.0 (each,a+b)/ 0.632 (a)/0.368 (b)	mStd Mix 1.0 ng/mL
mStd Mix 10 ng/mL	10 (each,a+b)/ 6.32 (a)/3.68 (b)	50	1000	0.50 (each,a+b)/ 0.316 (a)/0.184 (b)	mStd Mix 0.50 ng/mL
mStd Mix 1.0 ng/mL	1.0 (each,a+b)/ 0.632 (a)/0.368 (b)	100	1000	0.10 (each,a+b)/ 0.0632 (a)/0.0368 (b)	mStd Mix 0.10 ng/mL

#### 4) Preparation of Solvent Calibration Solutions for Evaluation of Matrix Effect

Solvent calibration solutions were prepared in methanol/water (80/20, v/v) fresh on the day of analysis by several dilution of the intermediate solvent solution.

A summary of the typical dilutions is presented in the following table:

**Table IX: Preparation of Calibration Solutions of IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990, and Pyrazole Carboxamide in Methanol/Water (80/20, v/v)**

Reference of standard solution used	Concentration (ng/mL)	Volume taken (µL)	Final volume (µL)	Final concentration (ng/mL)	Reference of standard solution produced
PA-0059-01-02	1000 (each,a+b)/ 632 (a)/368 (b)	100	1000	100 (each,a+b)/ 63.2 (a)/36.8 (b)	Std Mix 100 ng/mL (not injected)
Std Mix 100 ng/mL	100 (each,a+b)/ 63.2 (a)/36.8 (b)	100	1000	10 (each,a+b)/ 6.32 (a)/3.68 (b)	Std Mix 10 ng/mL
Std Mix 100 ng/mL	100 (each,a+b)/ 63.2 (a)/36.8 (b)	50	1000	5.0 (each,a+b)/ 3.16 (a)/1.84 (b)	Std Mix 5.0 ng/mL
Std Mix 100 ng/mL	100 (each,a+b)/ 63.2 (a)/36.8 (b)	25	1000	2.50 (each,a+b)/ 1.58 (a)/0.920 (b)	Std Mix 2.5 ng/mL
Std Mix 10 ng/mL	10 (each,a+b)/ 6.32 (a)/3.68 (b)	100	1000	1.0 (each,a+b)/ 0.632 (a)/0.368 (b)	Std Mix 1.0 ng/mL
Std Mix 10 ng/mL	10 (each,a+b)/ 6.32 (a)/3.68 (b)	50	1000	0.50 (each,a+b)/ 0.316 (a)/0.184 (b)	Std Mix 0.50 ng/mL

#### V. Laboratory Specimen Preparation

Specimen preparation was not necessary since the provided soil samples were already homogenized.

## VI. Sample Weights and Fortifications

Control (untreated) specimens of soil were fortified prior to extraction as described below. The solvent is allowed to evaporate before starting the extraction procedure. The analytes were fortified jointly.

**Table X: Sample Weights and Fortifications for Soil**

Fortified analytes	Matrix	Sample weight (g)	Reference of mixed fortification solution used	Concentration of fortification solution (ng/mL)	Volume of fortification solution added (µL)	Fortification level (each) (mg/kg)*
IR9792/F9990, 3-hydroxy-IR9792/F9990, 1-carboxy-IR9792/F9990 (sum of diastereomers), and Pyrazole Carboxamide	Soil	5.000 (±1%)	PA-0059-01-03	100	250	0.005 (LOQ)
			PA-0059-01-02	1000	250	0.050 (10xLOQ)

\*Based on dry weight.

## VII. Sample Work-Up Procedure

- Weigh **5.0 ± 0.1 g** of homogenized soil sample into a 50 mL plastic centrifuge tube.
- Fortify the control samples at the LOQ and 10xLOQ.
- Add 25 mL of acetone/water (9/1, v/v) to each centrifuge tube and place on a reciprocal shaker for 60 min at 300 excursions/minute.
- Centrifuge samples at 4000 rpm for 10 min.
- Decant the supernatant into a 100 mL volumetric flask. Use funnel with filter paper, if needed.
- Repeat extraction by adding 25 mL of acetone/water (1/1, v/v).
- Sonicate the samples at 40 °C for 60 min.
- Shaker on a reciprocal shaker for 60 min at 300 excursions/minute.
- Centrifuge samples at 4000 rpm for 10 min.
- Decant supernatant into the 100 mL volumetric flask already containing the first raw extract.
- Repeat extraction by adding 25 mL of acetone/(0.5 N HCl in water) (1/1, v/v).
- Sonicate the samples at 40 °C for 60 min.
- Shaker on a reciprocal shaker for 60 min at 300 excursions/minute.
- Centrifuge samples at 4000 rpm for 10 min.
- Decant supernatant into the 100 mL volumetric flask already containing the other two raw extracts.
- Fill up to the **100 mL (V<sub>Ex</sub>)** volume mark with acetone.
- Transfer 50 mL of the mixed extract into a new 50 mL centrifuge tube. The rest can be discarded.
- Centrifuge samples at 4000 rpm for 10 min.
- Using a transfer pipette, transfer a portion of the supernatant to a 15 mL centrifuge tube, up to the 5 mL graduation mark (**V<sub>1</sub> = 5 mL**).
- Place the sample under nitrogen evaporation in order to evaporate off the acetone. Approximately 1.5 mL of raw extract should be left.
- Remove from nitrogen evaporation, and bring back up to the **5 mL (V<sub>Final</sub>)** graduation mark with methanol.
- Shake well to mix.
- Transfer a portion to a HPLC vial for HPLC-MS/MS analysis.

### VIII. Chromatographic and Mass Spectrometric Conditions

A summary of the typical chromatographic and mass spectrometric conditions used for quantification is included in the following tables:

**Table XI: Summary of chromatographic and mass spectrometric conditions**

Chromatographic conditions				
HPLC system	Shimadzu Nexera X2 HPLC			
Column	Phenomenex Kinetex® 2.6 µm C18 100 Å, Cat. No. 00B-4462-E0, 50 mm x 4.6 mm, 2.6 µm			
Column oven temperature	20 °C			
Injection volume	10 µL			
Washing Solution	Methanol/HPLC Water (50/50, v/v)			
Mobile phases	Eluent A: HPLC Water + 10 mM ammonium acetate + 0.2 % formic acid Eluent B: Methanol + 0.2 % formic acid			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	90	10	600
	3.50	90	10	600
	6.00	60	40	600
	22.00	20	80	600
	23.00	20	80	600
	23.10	90	10	600
	24.00	90	10	600
Retention times	IR9792/F9990: ≈19.0 min 3-hydroxy-IR9792/F9990: ≈18.0 min 1-carboxy-IR9792/F9990 (Diastereomer a): ≈11.9 min 1-carboxy-IR9792/F9990 (Diastereomer b): ≈12.8 min Pyrazole carboxamide: ≈3.0 min			

Mass spectrometric conditions						
MS system	Applied Biosystems Sciex API 4000					
Ionisation type	Electrospray ionisation (ESI, Turbolon Spray)					
Polarity	Positive/negative ion modes					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V (1-carboxy-IR9792/F9990 and pyrazole carboxamide) 4500 V (IR9792/F9990) -4500 V (3-hydroxy-IR9792/F9990)	Ionspray turbo heater (TEM)			450 °C	
Curtain gas (CUR)	10 (arbitrary units)	Gas flow 1 (GS1)		60 (arbitrary units)		
Collision gas (CAD)	12 (arbitrary units)	Gas flow 2 (GS2)		60 (arbitrary units)		
Analyte monitored	Mass transition monitored ( <i>m/z</i> )	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
IR9792/F9990	352 → 332 <sup>#</sup>	106	10	23	8	250
	352 → 257	80	10	40	18	250
3-hydroxy-IR9792/F9990	366 → 175 <sup>#</sup>	-110	-10	-30	-11	250
	366 → 131	-93	-10	-40	-7	250
1-carboxy-IR9792/F9990 (Diastereomer a)	382 → 336.200 <sup>#</sup>	76	10	21	10	250
	382 → 296.100	76	10	35	20	250
1-carboxy-IR9792/F9990 (Diastereomer b)	382 → 336.201 <sup>#</sup>	76	10	21	10	250
	382 → 296.101	76	10	35	20	250
Pyrazole carboxamide	176 → 136 <sup>#</sup>	41	10	23	8	250
	176 → 156	41	10	13	10	250

<sup>#</sup> proposed (and/or used) for quantification but both of the mass transitions listed can be used for quantification.

## IX. Special Precautions

None

## X. Calculation of Results

Quantification was performed using calibration plots with six (6) different concentration levels covering the required calibration range.

A calibration curve was injected at the beginning and at the end of the analytical set. In addition, at least one calibration standard was injected after not more than eight (8) samples to check the response variability.

The linearity of the detection system was demonstrated by use of standard solutions covering a working range which is equivalent no more than 40 % of the LOQ and at least + 20 % of the highest analyte concentration level in any sample extract.

A linear regression was performed with 1/x weighting. The correlation coefficient (r) was greater or equal to 0.997.



A linear calibration function ( $y = a + b \cdot x$ ) as determined by software Analyst 1.6.2, SCIEX is used to calculate the analyte concentration in final extracts as follows:

$C_A =$	$\frac{A_A - a}{b}$
$C_A$	Concentration of analyte in final extract (ng/mL) (x)
$A_A$	Peak area of analyte in the final solution (counts) (y) as obtained by integration with software Analyst 1.6.2, SCIEX
a	y -axis Intercept of the calibration curve (counts)
b	Slope of calibration curve (counts)

The residues are calculated according to the following equations.

Residue in soil [mg/kg]:

$R =$	$\frac{C_a \cdot V_{Ex} \cdot V_{Final} \cdot V_1 \cdot W \cdot DF \cdot CF}{W}$
R	Analyte Residue (mg/kg)
$C_a$	Analysed concentration of the sample, as calculated from the matrix calibration function (ng/mL)
$V_{Ex}$	Extraction volume (100 mL)
$V_{Final}$	Final volume (5.0 mL)
$V_1$	Aliquot of the extract (5.0 mL)
W	Sample weight (g)
DF	Dilution factor (1)
CF	Conversion from ng into $\mu\text{g}$ (1000)

An example calculation for a recovery sample fortified with IR9792/F9990 (352  $m/z$  -> 332  $m/z$ ) at 0.005 mg/kg (Sample ID S01046; Soil 0.005-6) is presented below:

Analyte Residue (R) (mg/kg) =	$\frac{C_a \cdot V_{Ex} \cdot V_{Final} \cdot V_1 \cdot W \cdot DF \cdot CF}{W}$
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**Note:** Slight rounding differences may be noted when using a hand calculator. Use full computer/calculator precision in any intermediate calculations. Round only the final value.

**RECOVERY CALCULATION**

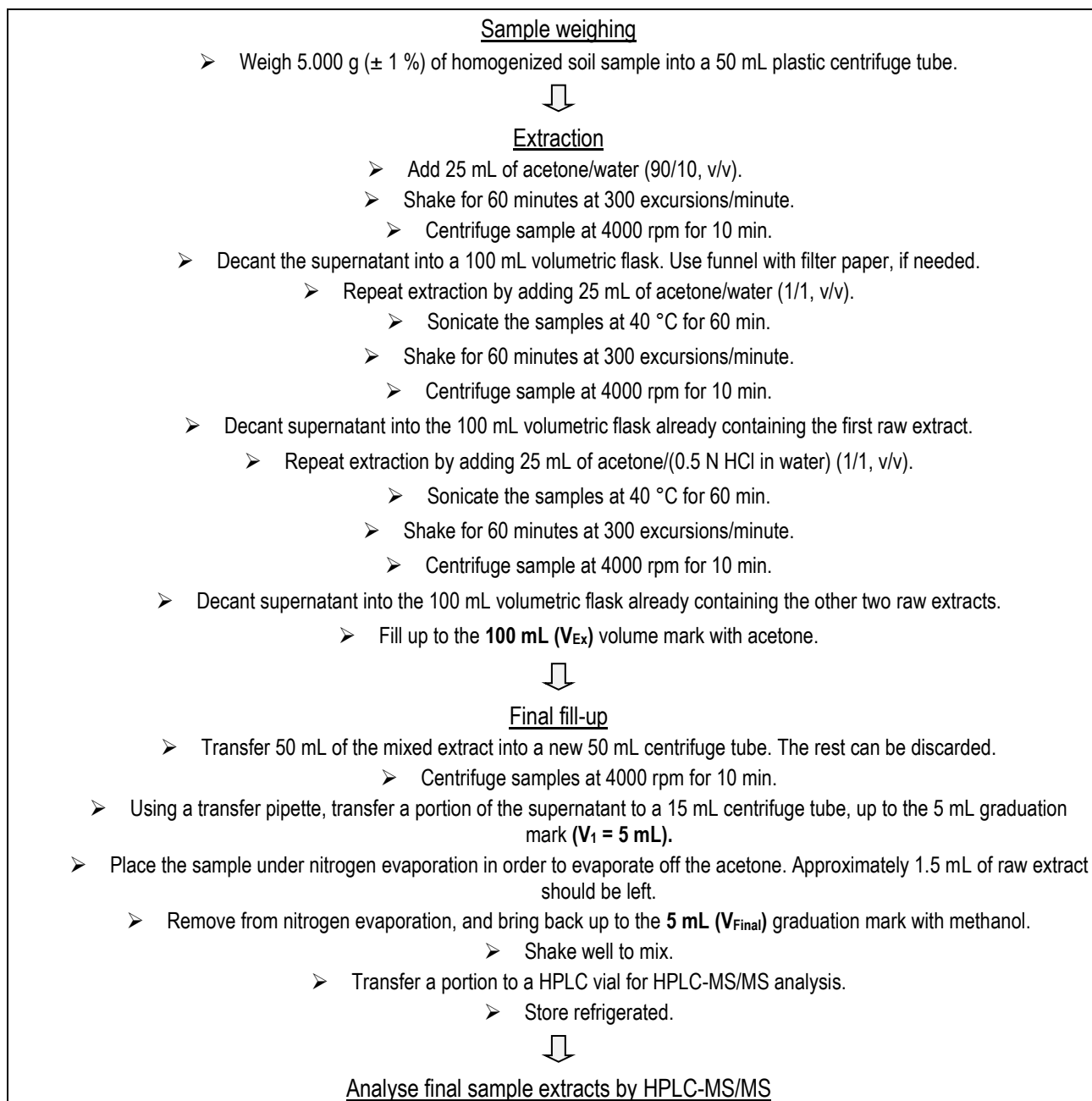
The recovery of a fortification experiment is calculated as follows:

Recovery (%) =	$\frac{R}{F} \times 100$
R	Analyte residue
F	Nominal fortification level

An example calculation for a recovery sample fortified with IR9792/F9990 (352 m/z -> 332 m/z) at 0.005 mg/kg (Sample ID S01046; Soil 0.005-6) is presented below:

Recovery Value (%) =	$\frac{\quad}{\quad} \times 100$
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## XI. Method Flow Chart



## XII. Safety Information

Reagents	H- and P-Codes	H- and P-Phrases
<u>Methanol</u>	H225 H301+H311+H331 H370 <hr/> P210 P280 P302+P352+P312 P304+P340+P311 P370+P378 P403+P235	<ul style="list-style-type: none"> <li>Highly flammable liquid and vapour.</li> <li>Toxic if swallowed, in contact with skin or if inhaled.</li> <li>Causes damage to organs</li> <li>Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.</li> </ul> <ul style="list-style-type: none"> <li>Wear protective gloves/protective clothing/eye protection/face protection.</li> </ul> <ul style="list-style-type: none"> <li>IF ON SKIN: Wash with plenty of soap and water, and call a POISON CENTER/doctor/... if you feel unwell.</li> <li>IF INHALED: Remove person to fresh air and keep comfortable for breathing and call a POISON CENTER/doctor/...</li> <li>In case of fire: use extinguishing powder or dry sand.</li> <li>Store in a well-ventilated place. Keep cool.</li> </ul>
<u>Acetone</u>	H225 H319 H336 EUH066 <hr/> P210 <hr/> P280 P305+P351+P338	<ul style="list-style-type: none"> <li>Highly flammable liquid and vapour.</li> <li>Causes serious eye irritation.</li> <li>May cause drowsiness or dizziness.</li> <li>Repeated exposure may cause skin dryness or cracking.</li> <li>Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.</li> </ul> <ul style="list-style-type: none"> <li>Wear protective gloves/protective clothing/eye protection/face protection.</li> </ul> <ul style="list-style-type: none"> <li>IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</li> </ul>
<u>Ammonium acetate</u>	H319 H315 H335 <hr/> P280 P302+P352 P304+P340 P305+P351+P338 P309+P311	<ul style="list-style-type: none"> <li>Causes serious eye irritation.</li> <li>Causes skin irritation.</li> <li>May cause respiratory irritation.</li> <li>Wear protective gloves/protective clothing/eye protection/face protection.</li> </ul> <ul style="list-style-type: none"> <li>IF ON SKIN: Wash with plenty of soap and water</li> <li>IF INHALED: Remove person to fresh air and keep comfortable for breathing.</li> <li>IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</li> <li>IF exposed or if you feel unwell: call a POISON CENTER or doctor/physician.</li> </ul>

Reagents	H- and P-Codes	H- and P-Phrases
<u>Hydrochloric acid</u>	H290 H314 H335 <hr/> P280  P305+P351+P338  P301+P330+P331  P308+P310	<ul style="list-style-type: none"> <li>• May be corrosive to metals.</li> <li>• Causes severe skin burns and eye damage.</li> <li>• May cause respiratory irritation.</li> <li>• Wear protective gloves/protective clothing/eye protection/face protection.</li>   <li>• IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</li> <li>• IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.</li>   <li>• IF exposed or concerned: Immediately call a POISON CENTER/doctor/...</li> </ul>
<u>Formic acid</u>	H314 <hr/> P280  P305+P351+P338  P301+P330+P331  P309+P311	<ul style="list-style-type: none"> <li>• Causes severe skin burns and eye damage.</li> <li>• Wear protective gloves/protective clothing/eye protection/face protection.</li>   <li>• IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</li> <li>• IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.</li>   <li>• IF exposed or if you feel unwell: call a POISON CENTER or doctor/physician.</li> </ul>