

INTRODUCTION

A residue method for the determination of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH in soil was developed and evaluated in this study. Method performance was discussed in terms of EU method parameters including accuracy, precision, limit of quantitation, specificity and linearity.

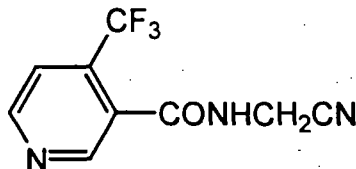
DOCUMENTS

This report summarizes the data acquired under Ricerca Protocols 12280-0 (Washington), 12281-0 (California), 12282-0 (North Dakota) and 12283-0 (North Carolina). These four studies were Residue Bareground Soil Dissipation Studies. The data generated in these studies is presented in this report as a validation of the soil method developed for the analysis of IKI-220 and its five soil metabolites.

TEST/REFERENCE SUBSTANCES

The structure, CAS registry number, and chemical name for IKI-220 and its metabolites are listed below.

- **IKI-220**



Chemical name: *N*-cyanomethyl-4-trifluoromethylnicotinamide

ISO name: Flonicamid (proposed)

CAS No.: 158062-67-0

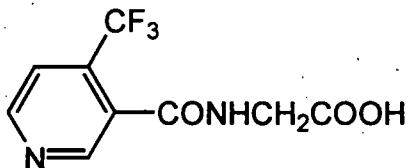
Molecular Wt.: 229.2

Lot Number: 9803

Purity: 99.7%

Expiration: November 11, 2003

• TFNG



Chemical names: *N*-(4-trifluoromethylnicotinoyl)glycine

CAS No.: 207502-65-6

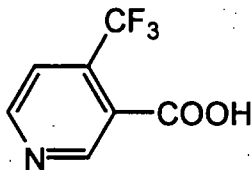
Molecular Wt.: 248.2

Lot Number: 0006

Purity: 99.4%

Expiration January 19, 2006

• TFNA



Chemical names: 4-trifluoromethylnicotinic acid

CAS No.: 158063-66-2

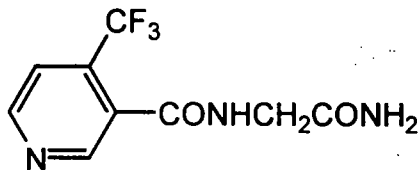
Molecular Wt.: 191.1

Lot Number: 0006

Purity: 99.4%

Expiration January 19, 2006

• TFNG-AM



Chemical names: *N*-(4-trifluoromethylnicotinoyl)glycinamide

CAS No.: 158062-96-5

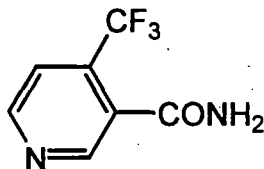
Molecular Wt.: 249.2

Lot Number: 0006

Purity: 99.5%

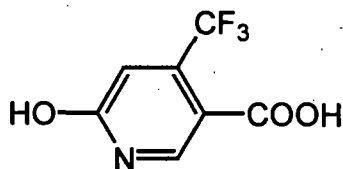
Expiration January 19, 2006

• TFNA-AM



Chemical names: 4-trifluoromethylnicotinamide
 CAS No.: 158062-71-6
 Molecular Wt.: 190.1
 Lot Number: 0006
 Purity: 100%
 Expiration: January 19, 2006

• TFNA-OH



Chemical names: 6-hydroxy-4-trifluoromethylnicotinic acid
 CAS No.: None
 Molecular Wt.: 207.1
 Lot Number: 0010
 Purity: 100%
 Expiration: January 19, 2006

OBJECTIVE

The objective of this study was to evaluate an analytical method for the determination of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM, and TFNA-OH residues in soil. This report takes the data and explains how the method is valid based on method accuracy, precision (repeatability), limit of quantitation, specificity and linearity. The method demonstrated successful recovery of IKI-220 and its metabolites from 5.0 – 50.0 ppb-fortifications in four different soil types.

ANALYTICAL PROCEDURE

Summary

IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM, and TFNA-OH were extracted from soil using an Accelerated Solvent Extractor (ASE). Twenty grams of soil were extracted with a solution of water/methanol (70/30; v/v) at a temperature of 110°C and a pressure of 150 bar, over a seven-minute static cycle. The final volume was adjusted to 40 mL with 70/30 water/methanol if necessary. The extract was analyzed by LC-MS/MS using an 8-point calibration curve prepared from standards. IKI-220 and TFNA-AM are determined using turbo ion spray injector with the detector in the positive ion mode. TFNG, TFNA, TFNG-AM, TFNA-OH are determined also with turbo ion spray, but with the detector in the negative ion mode.

Preparation of Standards

Approximately 3 to 5 mg of each of the bulk standards are weighed into separate 25 mL Class A volumetric flasks. The solutions are brought to volume with acetonitrile (HPLC Grade) to make individual standards at 120 to 200 µg/mL.

A portion of each of these stock solutions is transferred to an appropriately sized Class A volumetric and diluted with 30 % methanol in water (HPLC Grade) to make a combined solution containing each of the six standards at 20 µg/mL (e.g. 10 mL of a 200 µg/mL individual standard diluted to 100 mL).

All lower concentration standards are prepared are prepared from the 20 µg/mL stock mixed standard by diluting with 30% Methanol in water. Calibration solutions should be in the range of 0.1 to 0.001 µg/mL with five standards in the range to evaluate linearity on the LC-MS/MS.

Standards prepared in this manner should be stored in a freezer and should be stable for four months. New standards should be compared against the old to evaluate stability over time.

Extraction

Reagents

Methanol (MeOH)	HPLC Grade, or equivalent
H ₂ O	HPLC Grade, or equivalent
Extraction Solvent: Water/Methanol (70/30). Combine and mix 700 mL HPLC grade water, 300 mL HPLC grade methanol.	

Equipment

Accelerated Solvent Extractor ASE 200 Dionex Corporation

Analytical Balance

Cell Filters – Dionex p/n 049458

Disposable pipettes

Eppendorf adjustable pipettors, 100 μ L or 1,000 μ L or equivalent

Hydromatrix, Varian Corp.

Mettler BB-2400 balance or equivalent

VWR Scientific Multi-Tube Vortexer or equivalent

ASE Extraction Solution and Sample Volume Adjustment Solution:

Water/Methanol (70/30). Combine and mix 700 mL HPLC grade water, 300 mL HPLC grade methanol.

Procedure

1. A 20 gram sample of soil homogenate is mixed with 4 grams of hydromatrix and is placed into a 33 mL ASE extraction cell.
2. The ASE extractor is programmed for a run cycle and the samples are extracted. The following are the ASE 200 programmed criteria:
 - Heat cycle set at default (6 min).
 - Static cycle set at 7 minutes at 110° C and 150 Bar (2176 psi).
 - Preheat cycle is set to 0 minutes.
 - Purge time is set at 300 second.
 - Cell volume is set at 35 to 50% in order to give a final extract volume of 40 mL.
3. Following extraction, the sample volume was adjusted to 40 mL and a portion of the extract was placed in an HPLC vial for LC-MS/MS quantitation.

Quantitation

Instruments Used

PE Sciex LC-MS/MS Model 3000

Typical parameters on a PE Sciex API 3000 (the parameters may be modified for best results):

IS (Ion Source)	-4500 (negative ion) 5000 (positive ion)
TEM (Temperature)	500 C
MR Pause	5.0 ms
NEB (Nebulizer Gas, N ₂)	12.0
CUR (Curtain Gas, N ₂)	14.0
CAD (Collision Activated Dissociation Gas-N ₂)	3.0

Reagents Used

Methanol – Optima, Fisher
Acetic Acid-ACS Grade, Fisher
Water – HPLC Grade, Fisher

HPLC Conditions—Positive Ion Mode

Column:	LUNA, Phenyl-hexyl 150 x 2 mm, 5 μm
Injection volume:	5 μL
Oven temperature:	Column at room temperature
Mobile phase:	A: 1% acetic acid in water B: methanol
Run time:	5 min
Flow rate (column):	0.3 mL/min
Retention times:	
IKI-220:	approx. 4.53 min.
TFNA-AMi:	approx. 3.94 min.

HPLC-Gradient¹

Time [min]	0	2	2.5	2.6	5
% A	90	50	50	90	stop
% B	10	50	50	10	stop

¹The gradient was modified, as necessary, to optimize the separation.

MS/MS Conditions - Positive Ion Mode

Ionization Mode: Turbo IonSpray® Positive Ion Mode
 Scan Mode: Multiple Reaction Monitoring (MRM)
 Precursor → Product Ion Pair
 IKI-220 230.0 → 202.9
 TFNA-AM 191.0 → 148.0

HPLC Conditions—Negative Ion Mode

Column: LUNA, Phenyl-hexyl 150 x 2 mm, 5 µm
 Injection volume: 5 µL
 Oven temperature: Column at room temperature
 Mobile phase: A: 1% acetic acid in water
 B: methanol
 Run time: 8 min
 Flow rate (column): 0.3 mL/min
 Retention times:
 TFNG: approx. 6.68 min.
 TFNG-AM: approx. 5.52 min.
 TFNA: approx. 7.29 min.
 TFNA-OH: approx. 7.12 min.
 HPLC-Gradient¹

Time [min]	0	6	6.1	8
% A	95	50	95	stop
% B	5	50	5	stop

¹The gradient was modified, as necessary, to optimize the separation.

MS/MS Conditions - Negative Ion Mode

Ionization Mode: Turbo IonSpray® Negative Ion Mode
 Scan Mode: Multiple Reaction Monitoring (MRM)
 Precursor → Product Ion Pair
 TFNG-AM 246.3 → 99.0
 TFNA-OH 206.1 → 162.0
 TFNG 247.3 → 183.0
 TFNA 190.3 → 145.8

METHOD MODIFICATION

The mobile phases and gradient profiles for the LC-MS/MS were altered from the conditions listed in protocol amendments 1 to each of the four protocols. The mobile phase was switched from an acetonitrile/water with 1% formic acid gradient to a methanol/water gradient with 1% acetic acid added as a pH modifier.

This method modification had no adverse affect on the study, as concurrent recoveries were run and validated with every sample set.

CALCULATION OF RESIDUES

IKI-220 and metabolite residues were quantitated using linear multi-point calibration curves generated from the injection of external standards. Detailed sample calculations are found in Appendix A.

Figure 1: Flow Diagram for IKI-220 Soil Method

