FINAL REPORT

Independent Laboratory Validation of Residue Analytical Method for Determination of NF-180, QP-1-1 and QP-1-7 in Soil and NF-180 in Sediment

GLP Study Number
BPL19-0009

Study Completion Date
11 Jul 2019

Guidelines
ENV/JM/MONO(2007)17, OECD
SANCO/825/00 rev. 8.1
OCSPP 860.1340
OCSPP 850.6100

1. STUDY OBJECTIVES

This study was conducted in compliance with the principles of Good Laboratory Practices (GLP). The objective was to perform an independent laboratory validation of the method for determination of NF-180, QP-1-1 and QP-1-7 in soil and the method for determination of NF-180 in sediment according to the guidance documents OCSPP850.6100, OPPTS860.1340 and SANCO/825/00 rev. 8.1.

Analysis	**LOQ**	**Method**
NF-180, QP-1-1 and QP-1-7 in soil	0.002 mg/kg	HPLC-MS/MS
NF-180 in sediment	0.05 mg/kg	HPLC-MS/MS

HPLC-MS/MS: High Performance Liquid Chromatography coupled with tandem Mass Spectrometry detection.

2. GENERAL INFORMATION

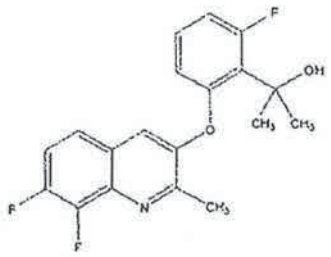
2.1. Guidelines

Technical	- Guidance document on pesticide residue analytical methods, ENV/JM/MONO(2007)17, OECD - SANCO/825/00 rev. 8.1 - OCSPP 860.1340 - OCSPP 850.6100
GLP	- French regulation: Annexe II à l'article D523-8 du Code de l'Environnement, 16 Oct. 2007. - C(97)186/FINAL - Council Decision Amending Annex II To The Council Decision Concerning The Mutual Acceptance Of Data In The Assessment Of Chemicals [C(81)30(FINAL)] - Directive (EC) N°2004/10 of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version) - The OECD Principles of Good Laboratory Practice (as Revised in 1997), OECD Series on Principles of GLP and Compliance Monitoring Number 1, ENV/MC/CHEM(98)17

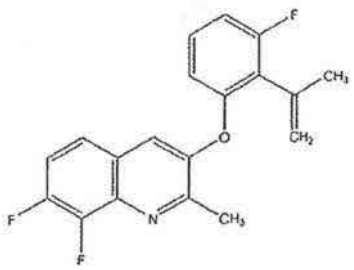
3. MATERIAL AND METHODS

3.1. Test / Reference Item

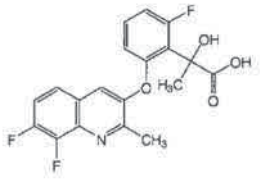
These test / reference items were used for calibration and fortification purposes.

Reference Item 1	
Common name	**NF-180**
Chemical name	2-[2-(7,8-difluoro-2-methylquinolin-3-yloxy)-6-fluorophenylpropan-2-ol
Molecular formula	$C_{19}H_{16}F_3NO_2$
Molecular weight	347.33
Structure	
Supplier	Nisso Chemical Analysis Service Co., Ltd.
Batch No.	TRED-001
Purity	99.1%
Expiration date	06 Jul 2021
Storage conditions	<-18°C*

*"Keep in a freezer mention on certificate of analysis is considered equivalent to <-18°C

Reference Item 2	
Common name	**QP-1-1**
Chemical name	7,8-difluoro-3-(3-fluoro-2-isopropenylphenoxy)-2-methylquinoline
Molecular formula	$C_{19}H_{14}F_3NO$
Molecular weight	329.32
Structure	
Supplier	Nisso Chemical Analysis Service Co., Ltd.
Batch No.	18215-D. SATO
Purity	99.9%
Expiration date	07 Mar 2020
Storage conditions	<-18°C*

*"Keep in a freezer (at -20±10 °C)" mention on certificate of analysis is considered equivalent to <-18°C

Reference Item 3	
Common name	**QP-1-7**
Chemical name	2-[2-(7.,8-difluoro-2-methylquinolin-3-yloxy)-6-fluorophenyl]-2-hydroxypropanoic acid
Molecular formula	$C_{19}H_{14}F_3NO_4$
Molecular weight	377.31
Structure	
Supplier	Nisso Chemical Analysis Service Co., Ltd.
Batch No.	31-17149-T.AIHARA
Purity	98.6%
Expiration date	26 July 2019
Storage conditions	<-18°C*

*"Keep ~~at~~ **in** a freezer (at -20±10 °C)" mention on certificate of analysis is considered equivalent to <-18°C

When stability data and storage conditions were not available, working solutions were freshly prepared.
Usual safety precautions with chemicals were obeyed.

All specifications given on the certificate of analysis, provided by the sponsor/ supplier, are essential for correct identification of the test / reference item for use under GLP. They were not verified by the test facility and no claim of GLP compliance were made for these data, except where this is explicitly claimed on the certificate of analysis. Additional specifications for test / reference item characterisation may originate from (non-GLP) sources other than the sponsor/ supplier and are also part of the final report.

3.2. Test System

Table 1: Test System

Test System	Preparation	Origin	Identification
Soil	Homogenization using knife mill.	Commercial supplier: Lufa speyer	ECH18-0006
Sediment	Homogenization by hand followed by centrifugation at 2800 g for 15 min to separate interstitial water. Water content after centrifugation: 26.8 %*	Commercial supplier Fraunhofer	ECH19-00012

*Non GLP data generated by SGS France – Laboratoire de Rouen

Characterisation of test system is shown in appendix
Test systems were stored frozen (<-18 °C) until fortification.

3.3. Method for Determination of NF-180, QP-1-1 and QP-1-7 in Soil

3.3.1. Principle

This method was validated in soil according to SANCO/3029/99 rev. 4, SANTE/11945/2015 and OPPTS860.1340 under GLP study 160694 (RD-10423) (Ref. 1).

Residues of NF-180, QP-1-1 and QP-1-7 were extracted twice via shaking in a solution of acetonitrile/water/acetic acid (80/20/5; v/v/v). Extracts were gravity filtered and brought up to a known volume with water. Aliquots of the extracts were diluted with a solution of acetonitrile/water (50/50; v/v), syringe-filtered and analyzed via LC-MS/MS with electrospray ionization.

The limit of quantification is 0.002 mg/kg for each analyte and the limit of detection is 0.0005 mg/kg.

3.3.2. Equipment

Equivalent equipment may be used.

Table 2: Equipment

Equipment	Supplier	Model
Scale (precision 0.005mg)	Mettler-Toledo	XPE206DR
Scale (precision 0.001g)	Mettler-Toledo	XP2003SDR
Automatic Pipette	Thermo	Finnpipette
Ultrasonic bath	VWR	USC300TH
Laboratory Centrifuge	Haraeus	MEGAFUGE40R
Laboratory glassware volumetric Class A	VWR	Various
Paper filter	Whatman	589/1 125 mm ref. 10 300 011
PTFE filter	Sigma Aldrich	Acrodisc PTFE fliter 0.2 µm
Shake on Platform Shaker	Bioblock	SM30B
HPLC Column	Phenomenex	Kinetex 2.6 µm C18, 100 x 3.0 mm, 100 Å , Part. No. 00D-4462-Y0
LC Autosampler	Shimadzu	SIL-30ACMP
LC Pump	Shimadzu	LC-30AD
LC Solvent/degazing rack	Shimadzu	DGU-20A5R
LC Oven	Shimadzu	CTO-20AC
LC Reservoir Selection Valves	Shimadzu	FCV-11AL
Detector	AB Sciex	API5500 Qtrap
Software	AB Sciex	Analyst 1.6.2

3.3.3. Reagents

Equivalent reagents may be used.

Table 3: Reagents

Reagent	Purity/ Grade	Supplier
HPLC water	Water for UHPLC-MS	Carlo Erba
Acetonitrile	HyperSolv Chromanorm for HPLC	VWR
Acetic acid	AnalaR Normapur	VWR

Preparation
Mobile Phase "A" for HPLC analysis : 0.1% Acetic acid in water (v/v)
To prepare 1 L: Add 1 mL of concentrated acetic acid to approximately 900 mL of water in a volumetric flask. Bring up to a volume of 1 L with water. Cap and mix thoroughly. Degas at least 30 min in an ultrasonic bath. Should not be stored more than 1 week at room temperature in a closed flask.

Mobile Phase "B" for HPLC analysis : 0.1% Acetic acid in acetonitrile (v/v)
To prepare 1 L: Add 1 mL of concentrated acetic acid to approximately 900 mL of acetonitrile in a volumetric flask. Bring up to a volume of 1 L with acetonitrile. Cap and mix thoroughly. Degas at least 30 min in an ultrasonic bath. Should not be stored more than 1 week at room temperature in a closed flask.

Acetonitrile/water/acetic acid (80:20:5, v/v/v)
To prepare 1 L: Combine 800 mL of acetonitrile with 200 mL of HPLCgrade
water and 50 mL of acetic acid. Cap and mix thoroughly. Should not be stored more than 1 month at room temperature in a closed flask.

Acetonitrile/water (50:50, v/v)
To prepare 1 L: Combine 500 mL of acetonitrile with 500 mL of HPLCgrade
water. Cap and mix thoroughly. Should not be stored more than 1 month at room temperature in a closed flask.

Note: volumes can be adapted according needs.

3.3.4. Standard Solutions

3.3.4.1. Preparation of stock solutions

Weight approximately exactly 5 mg of NF-180, QP-1-1 and QP-1-7 separately into a 50 mL volumetric flask and filled up to the mark with the acetonitrile. Stock solution is approximately 0.1 mg/mL.
Two independent stock solutions (SM1 and SM2) are prepared to perform calibration and fortification solutions independently.
Exact concentrations are calculated taking into account exact weight and purity.

Stock solutions were proven to be stable for at least 117 days when stored frozen ≤-10°C (Ref.1).

3.3.4.2. Preparation of fortifications solutions

Fortification solutions were prepared as it described in the table below:

Table 4: Fortification solutions

Solution identification	Volume Taken (mL)	From solution	Dilution Solvent	Final volume (mL)	Concentration (ng/mL)
SF1 of SM2	0.4*	SM2	Acetonitrile	20	2000
SF2 of SM2	1	SF1 of SM2		10	200

* From SM2 of each analytes

3.3.4.3. Preparation of calibration solutions

Calibration solutions were prepared as it described in the table below:

Table 5: Standard solutions

Solution identification	Volume Taken (mL)	From solution	Dilution Solvent	Final volume (mL)	Concentration (ng/mL)
SF1 of SM1	0.1*	SM1	Acetonitrile/water (50:50, v/v)	10	1000
SF2 of SM1	1.0	SF1 of SM1		20	50
SF3 of SM1	0.5	SF1 of SM1		20	25
SF4 of SM1	0.1	SF1 of SM1		10	10
SF5 of SM1	0.05	SF1 of SM1		10	5
SF6 of SM1	0.05	SF1 of SM1		20	2.5
SF7 of SM1	0.05	SF1 of SM1		50	1
SF8 of SM1	0.1	SF2 of SM1		20	0.25

* From SM1 of each analytes

Standards solutions were diluted 10-folds in acetonitrile/water (50:50, v/v) or in control specimen final extract to prepare the standard calibration curve in solvent or in matrix at the following concentrations:

0.025 – 0.1 – 0.25 – 0.5 – 1.0 – 2.5 – 5.0 ng/mL

Equivalent to :

0.0005 – 0.002 – 0.005 – 0.01 – 0.02 – 0.05 – 0.1 mg/kg

3.3.5. Analytical Procedure

3.3.5.1. Specimens fortification

Fortifications were performed on control specimens using fortification solutions as follow:

Table 6: Fortifications

Fortification level (mg/kg)	Sample Weight (g)	Fortification solution identification	Spiking volume (mL)
0.002	20	SF2 of SM2 (200 ng/mL)	0.2
0.02	20	SF1 of SM2 (2000 ng/mL)	0.2

3.3.5.2. Specimens preparation

- Weigh about 20 g of homogenized specimen into a 250 mL plastic centrifuge tube. Sample fortification, if required, had to be carried out at this point. At least one untreated control and two control samples fortified with a known amount of NF-180, QP-1-1 and QP-1-7 should be analysed in each analytical set to demonstrate acceptable performance of the method.

Extraction

- Add 100 mL of acetonitrile/water/acetic acid (80:20:5, v/v/v) to the 20-g subsample.
- Shake on Platform Shaker for 30 minutes at approximately 200 rpm.
- Centrifuge sample at a setting of 3000 rpm and 5 minutes.

- Decant the supernatant extract through a filter paper into a 250-mL mixing cylinder.
- Extract soil a second time with 50 mL of acetonitrile/water/acetic acid (80:20:5, v/v/v).
- Shake on Platform Shaker for 30 minutes at approximately 200 rpm.
- Centrifuge sample at a setting of 3000 rpm and 5 minutes.
- Decant the supernatant liquid from the second extraction through the filter paper into the 250-mL mixing cylinder.
- Bring up sample extract to a final volume of 200 mL with water.

Final Dilution

- Transfer 5-mL of extract to a labeled 15-mL plastic centrifuge tube.
- Bring up to a final volume of 10 mL with acetonitrile/water (1:1, v/v).
- Filter an aliquot of the final extract through a 0.2-µm PTFE filter into an autosampler vial and analyze samples by LC-MS/MS.

Note: 0.2 µm filters were used intead of 0.45 µm. this modification was considered as a minor change without any impact on the validity of the ILV.

3.3.5.3. LC-MS/MS Analysis

Chromatographic Conditions			
Injection:	10 µL		
Mobile Phase A: Mobile Phase B:	0.1% acetic acid in water 0.1% acetic acid in acetonitrile		
Flow Rate:	0.5 mL/min		
Column	Phenomenex Kinetex 2.6 µm C18, 100 x 3.0 mm, 100 Å, Part. No. 00D-4462-Y0		
Autosampler temperature	10°C		
Column oven temperature	40°C		
Gradient	Time (min)	Phase A (%)	Phase B (%)
	0.00 0.15 5.00 5.90 6.00 7.00	70 70 5 5 70 70	30 30 95 95 30 30
Retention Times			
NF-180 QP-1-1 QP-1-7		Approx. 4.1 min Approx. 5.2 min Approx. 2.9 min	

Source Parameters	
Ion Source:	Turbo Ion Spray (ESI)
Ion Polarity	Positive
Curtain gas	30
Collision gas	Medium
Ionspray voltage	5500 V
Temperature	500°C
Ion source gas 1	50
Ion source gas 2	60

Acquisition Parameters						
Analytes	Mass transition (m/z)	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
NF-180	348.0 → 330.0*	91	10	31	26	200
	348.0 → 180.0	91	10	39	10	200
QP-1-1	330.0 → 180.0*	51	10	33	10	150
	330.0 → 314.0	51	10	45	16	150
QP-1-7	378.0 → 332.0*	126	10	29	24	150
	378.0 → 314.0	126	10	43	22	150

* proposed for quantification

Note: No guard column was used. This modification was considered as a minor change without any impact on the validity of the ILV.

3.3.6. Method Management and Time Requirements

Overnight and over a weekend stopping points

The analytical procedure may be interrupted overnight and, if necessary even over a weekend after the following step:

1. Final dilution

Procedural recoveries results validate storage step.

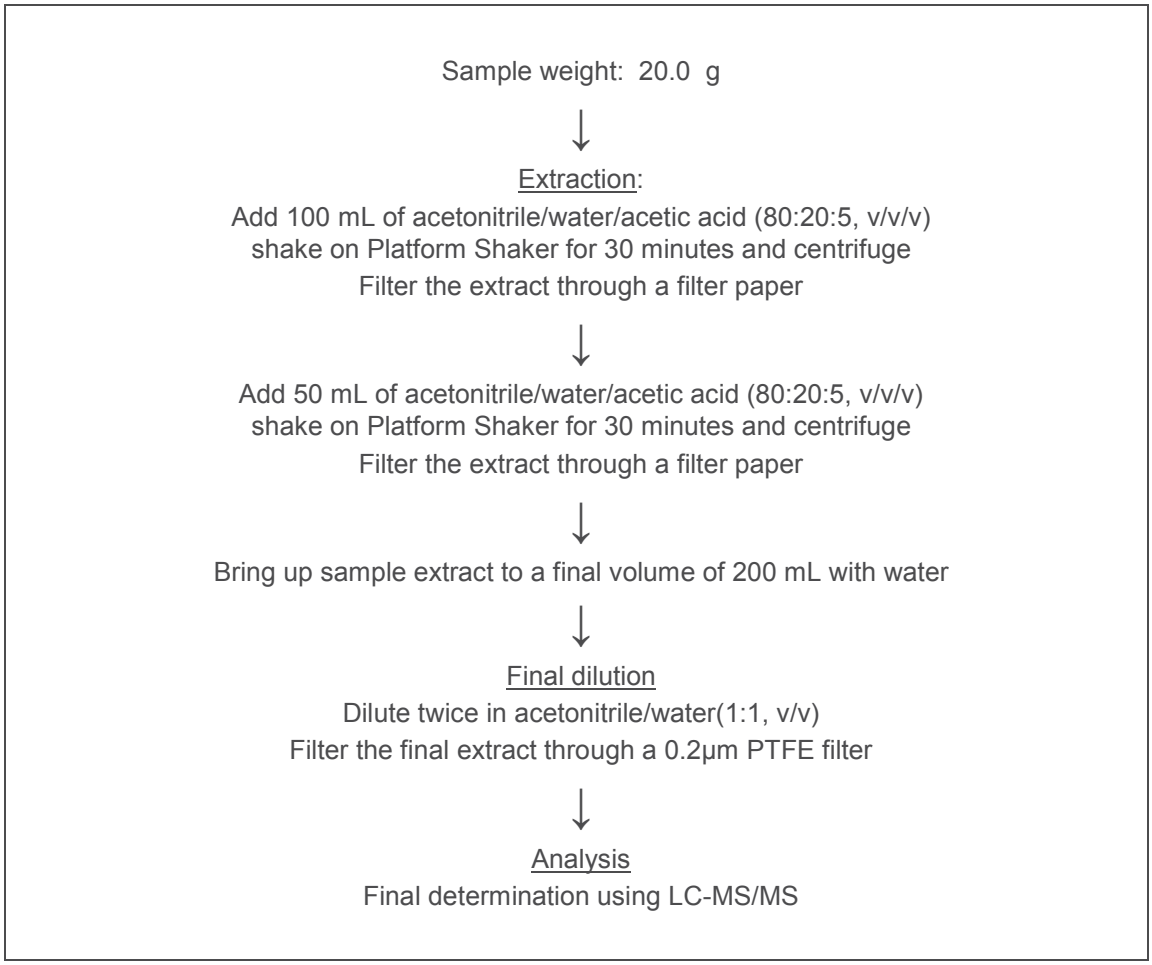
Recommended time management

1st day:	until end
Overnight	LC/MS/MS
2nd day:	calculation of results

Time Requirements

The analysis of one set of samples (= 30 samples, 1 control and 2 fortifications for recovery experiments) requires approximately 24 hours. This time includes the calculation of the results, the setup of the equipment if no special problems arise.

3.3.7. Flow chart



3.3.8. Expression of results

Amount of analyte in specimen is calculated as follow:

Calibration curve equation: y = a x + b, 1/x weighted,

- y: Analyte area
- x: Analyte conc

$$\text{Analyte amount (mg/kg)} = c \times \frac{V_{Ext} \times V_{fin}}{W \times V_A} \times \frac{1}{f} \times D$$

- c: Analyte concentration (ng/mL)
- V_{Ext}: Extraction volume (200 mL)
- V_A: Aliquot volume (5 mL)
- V_{fin}: Final volume (10 mL)
- W: Sample Weight (target 20 g)
- f: Conversion factor between ng and µg (1000)
- D: Dilution or concentration factor of the extract (if needed)

Recovery Calculation

$$\text{Recovery (\%)} = 100 \times \frac{\text{Analyte amount}_{\text{recovered}}}{\text{Analyte amount}_{\text{fortified}}}$$

3.3.9. Data Recording

The application programs used to acquire and derive data for this study include Word, Excel and Analyst.

Excel sheets used for this study were verified.

3.4. Method for Determination of NF-180 in Sediment

3.4.1. Principle

This method was validated in sediment according to SANCO/3029/99 rev. 4 and OCSPP 850.6100 under GLP study 12681.6134 (RD-10513) (Ref. 2).

Residues of NF-180 were extracted twice with acetonitrile/water/formic acid (90/10/0.1; v/v/v). After dilution in acetonitrile/ water (20/80; v/v), all samples were analyzed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

The limit of quantification is 0.05 mg/kg (on dry soil) and the limit of detection is 0.0125 mg/kg.

3.4.2. Equipment

Equivalent equipment may be used.

Table 7: Equipment

Equipment	Supplier	Model
MilliQ water system producer	Millipore	ACADEMIC A10
Scale (precision 0.005mg)	Mettler-Toledo	XPE206DR
Scale (precision 0.001g)	Mettler-Toledo	XP2003SDR
Automatic Pipette	Thermo	Finnpipette
Ultrasonic bath	VWR	USC300TH
Laboratory Centrifuge	Haraeus	MEGAFUGE40R
Laboratory glassware volumetric Class A	VWR	Various
Shake on Platform Shaker	Bioblock	SM30B
HPLC Column	Supelco	Ascentis Express C18, 2.7 µm, 2.1 × 100 mm, Part. No. 53823-U
LC Autosampler	Shimadzu	SIL-30ACMP
LC Pump	Shimadzu	LC-30AD
LC Solvent/degazing rack	Shimadzu	DGU-20A5R
LC Oven	Shimadzu	CTO-20AC
LC Reservoir Selection Valves	Shimadzu	FCV-11AL
Detector	AB Sciex	API5500 Qtrap
Software	AB Sciex	Analyst 1.6.2

3.4.3. Reagents

Equivalent reagents may be used.

Table 8: Reagents

Reagent	Purity/ Grade	Supplier
HPLC water	Water for UHPLC-MS	Carlo Erba
Acetonitrile	HyperSolv Chromanorm for HPLC	VWR
Formic acid	Emsure ACS	Merck

Preparation
Mobile Phase "A" for HPLC analysis : 0.1% Formic acid in water (v/v)
To prepare 1 L: Add 1 mL of concentrated formic acid to approximately 900 mL of water in a volumetric flask. Bring up to a volume of 1 L with water. Cap and mix thoroughly. Degas at least 30 min in an ultrasonic bath. Should not be stored more than 1 week at room temperature in a closed flask.

Mobile Phase "B" for HPLC analysis : 0.1% Formic acid in acetonitrile (v/v)
To prepare 1 L: Add 1 mL of concentrated formic acid to approximately 900 mL of acetonitrile in a volumetric flask. Bring up to a volume of 1 L with acetonitrile. Cap and mix thoroughly. Degas at least 30 min in an ultrasonic bath. Should not be stored more than 1 week at room temperature in a closed flask.

Acetonitrile/water/formic acid (90/10/0.1, v/v/v)
To prepare 1 L: Combine 900 mL of acetonitrile with 100 mL of HPLC grade water and 1 mL of formic acid. Cap and mix thoroughly. Should not be stored more than 1 month at room temperature in a closed flask.

Acetonitrile/ water (20/80; v/v) liquid reagent solution was prepared by
To prepare 1 L: Combine 200 mL of acetonitrile with 800 mL of HPLC grade water. Cap and mix thoroughly. Should not be stored more than 1 month at room temperature in a closed flask.

Note: volumes can be adapted according needs.

3.4.4. Standard Solutions

3.4.4.1. Preparation of stock solutions

Weight approximately exactly 5 mg of NF-180 into a 50 mL volumetric flask and filled up to the mark with the acetonitrile. Stock solution is approximately 0.1 mg/mL.
Two independent stock solutions (SM1 and SM2) are prepared to perform calibration and fortification solutions independently.
Exact concentrations are calculated taking into account exact weight and purity.

3.4.4.2. Preparation of fortifications solutions

Fortification solutions were prepared as it described in the table below:

Table 9: Fortification solutions

Solution identification	Volume Taken (mL)	From solution	Dilution Solvent	Final volume (mL)	Concentration (ng/mL)
SF1 of SM2	1	SM2	Acetonitrile	10	10 000
SF2 of SM2	1	SF1 of SM2		10	1000

3.4.4.3. Preparation of calibration solutions

Calibration solutions were prepared as it described in the table below:

Table 10: Standard solutions

Solution identification	Volume Taken (mL)	From solution	Dilution Solvent	Final volume (mL)	Concentration (ng/mL)
SF1 of SM1	0.2	SM1	Acetonitrile/ water (20/80; v/v)	20	1000
SF2 of SM1	1	SF1 of SM1		10	100
SF3 of SM1	0.2	SF2 of SM1		20	1
SF4 of SM1	0.16	SF2 of SM1		20	0.8
SF5 of SM1	0.06	SF2 of SM1		10	0.6
SF6 of SM1	0.04	SF2 of SM1		10	0.4
SF7 of SM1	0.1	SF2 of SM1		50	0.2
SF8 of SM1	0.05	SF2 of SM1		50	0.1
SF9 of SM1	0.05	SF2 of SM1		100	0.05

Standards solutions were diluted 10-folds in acetonitrile/ water (20/80; v/v) or in control specimen final extract to prepare the standard calibration curve in solvent or in matrix at the following concentrations:

0.005 – 0.01 – 0.02 – 0.04 – 0.06 – 0.08 – 0.1 ng/mL

Equivalent to :

0.0125 - 0.025 – 0.05 – 0.1 – 0.15 -0.2 - 0.25 mg/kg in sediment

3.4.5. Analytical Procedure

3.4.5.1. Specimens fortification

Fortifications were performed on control specimens using fortification solutions as follow:

Table 11: Fortifications

Fortification level (mg/kg)	Sample Dry Weight Equivalent (g)	Fortification solution identification	Spiking volume (mL)
0.05	5	SF2 of SM2 (1000 ng/mL)	0.25
0.5	5	SF1 of SM2 (10000 ng/mL)	0.25

3.4.5.2. Specimens preparation

- Weigh about 5 g of dry sediment equivalent of homogenized specimen into a 250 mL plastic centrifuge tube. Sample fortification, if required, had to be carried out at this point. At least one untreated control and two control samples fortified with a known amount of NF-180 should be analysed in each analytical set to demonstrate acceptable performance of the method.

Extraction

- Add 20 mL of acetonitrile/water/formic acid (90/10/0.1, v/v/v) to the subsample.
- Shake on Platform Shaker for 30 minutes at approximately 150 rpm.
- Centrifuge sample at a setting of 3000 rpm and 10 minutes.
- Decant the supernatant extract into a 50-mL volumetric flask.
- Extract soil a second time with 20 mL of acetonitrile/water/formic acid (90/10/0.1, v/v/v).
- Shake on Platform Shaker for 30 minutes at approximately 150 rpm.
- Centrifuge sample at a setting of 3000 rpm and 10 minutes.
- Decant the supernatant extract into the 50-mL volumetric flask.

- Bring up sample extract to a final volume of 50 mL with acetonitrile/water/formic acid (90/10/0.1, v/v/v).

Final Dilution
- Dilute 0.04-mL of extract into 10 mL of acetonitrile/water (20/80, v/v)
- Transfer into an autosampler vial and analyze samples by LC-MS/MS.

Note: Recoveries at 10 x LOQ were diluted transferring 3 mL of final extract into a 10 mL volumetric flask and the volume was brought to 10 mL using final extract of a control specimen.

3.4.5.3. LC-MS/MS Analysis

Chromatographic Conditions			
Injection:	10 µL		
Mobile Phase A: Mobile Phase B:	0.1 % formic acid in water 0.1 % formic acid in acetonitrile		
Flow Rate:	0.4 mL/min		
Column	Supelco Ascentis Express C18, 2.7 µm, 2.1 × 100 mm, Part. No. 53823-U		
Autosampler temperature	10°C		
Column oven temperature	40°C		
Gradient	Time (min)	Phase A (%)	Phase B (%)
	0.0 0.5 6.0 7.0 7.1 8.5	95 90 0 0 95 95	5 10 100 100 5 5
Retention Times			
NF-180		Approx. 4.3 min	
Source Parameters			
Ion Source:	Turbo Ion Spray (ESI)		
Ion Polarity	Positive		
Curtain gas	30		
Collision gas	Medium		
Ionspray voltage	5500 V		
Temperature	500°C		
Ion source gas 1	50		
Ion source gas 2	60		

Acquisition Parameters						
Analytes	Mass transition (m/z)	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
NF-180	348.0 → 330.1*	51	10	31	18	200
	348.0 → 180.0	51	10	39	22	200

* proposed for quantification

Note 1: No guard column was used. This modification was considered as a minor change without any impact on the validity of the ILV.
Note 2: Minor modification compared to validation report of injection temperature (Ref.2) (initially 5°C) considered to have no impact on the study.

3.4.6. Method Management and Time Requirements

Overnight and over a weekend stopping points

The analytical procedure may be interrupted overnight and, if necessary even over a weekend after the following step:

1. Final dilution

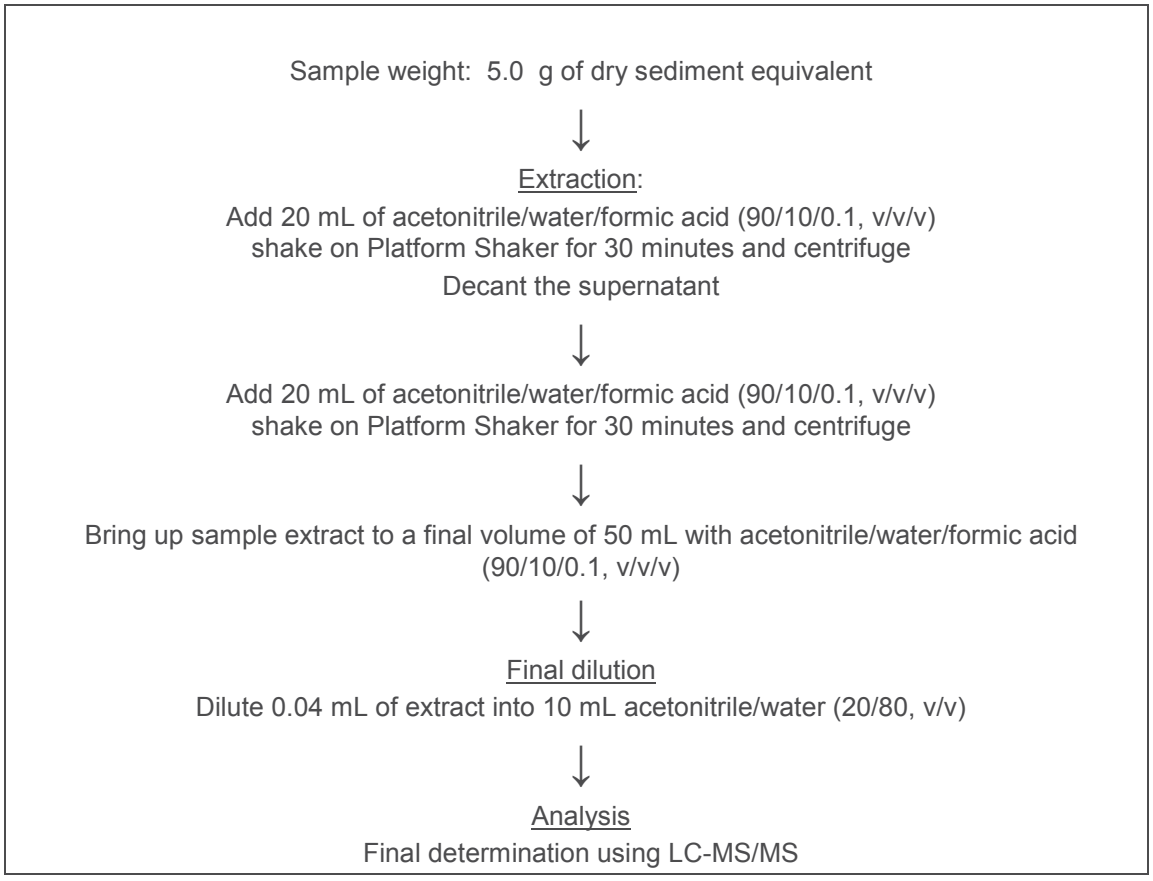
Recommended time management

1st day:	until end
Overnight	LC/MS/MS
2nd day:	calculation of results

Time Requirements

The analysis of one set of samples (= 30 samples, 1 control and 2 fortifications for recovery experiments) requires approximately 24 hours. This time includes the calculation of the results, the setup of the equipment if no special problems arise.

3.4.7. Flow chart



3.4.8. Expression of results

Amount of analyte in specimen is calculated as follow:

Calibration curve equation: y = a x + b, 1/x weighted,

- y: Analyte area
- x: Analyte conc

$$Analyte\ amount\ (mg/kg)\ =\ c \times \frac{V_{Ext} \times V_{fin}}{W \times V_A} \times \frac{1}{f} \times D$$

- c: Analyte concentration (ng/mL)
- V_{Ext}: Extraction volume (50 mL)
- V_A: Aliquot volume (0.04 mL)
- V_{fin}: Final volume (10 mL)
- W: Sample Weight (target 5 g)
- f: Conversion factor between ng and µg (1000)
- D: Dilution or concentration factor of the extract (if needed)

Recovery Calculation

$$\text{Recovery (\%)} = 100 \times \frac{\text{Analyte amount}_{\text{recovered}}}{\text{Analyte amount}_{\text{fortified}}}$$

3.4.9. Data Recording

The application programs used to acquire and derive data for this study include Word, Excel and Analyst.
Excel sheets used for this study were verified.

4. DEVIATIONS

No deviation from the study plan and from SGS France – Laboratoire de Rouen standard operating procedures (SOP) was observed.

5. RESULTS AND DISCUSSION

5.1. Method for Determination of NF-180, QP-1-1 and QP-1-7 in Soil

5.1.1. Selectivity

NF-180, QP-1-1 and QP-1-7 were analysed using LC-MS/MS considered as a highly selective method monitoring two specific transitions.
The evaluated transitions have been verified. Ion scan figure is shown in appendix.

5.1.2. Specificity

Two (2) control samples of soil were analysed to investigate residue level and to check for any background interferences at the expected retention time. In addition, at least one (1) reagent blank (sample extraction procedure without matrix) was run within the validation set.

For both mass transitions, control samples showed no significant interferences at the retention time of NF-180, QP-1-1 and QP-1-7 in soil; therefore, the specificity of the method is sufficiently proven.

5.1.3. Matrix Effects

Matrix effect was assessed by mean response factor (RF) comparison of matrix matched standards and standards solutions. Matrix effect was calculated as follow and a correction of blank value was applied where appropriate:

$$Matrix\ Effect(\%)\quad \frac{RF_{(Matrix\ Matched\ standards)} - RF_{(Solvent\ standards)}}{RF_{(Solvent\ standards)}} \times 100$$

Table 12: Matrix effect in soil

Analytes	Transition (m/z)	Soil
NF-180	348.0 → 330.0 Quantification)	-2.2 %
	348.0 → 180.0 Confirmation)	-0.4 %
QP-1-1	330.0 → 180.0 Quantification)	-6.6 %
	330.0 → 314.0 Confirmation)	-3.4 %
QP-1-7	378.0 → 332.0* Quantification)	-2.4 %
	378.0 → 314.0 Confirmation)	-3.1 %

Response factor RSD was below 20%

Even if non-significant matrix effect (< ±20%) on signal was found, quantification using matrix matched standards was performed.

5.1.4. Linearity

The linearity of the response was demonstrated by single determination of matrix-matched standards calibration on at least 5 concentration levels ranging from 0.025 ng/mL to 5 ng/mL (corresponding to 0.0005 mg/kg to 0.1 mg/kg in soil) for analysis of NF-180, QP-1-1, and QP-1-7. This covers the range from no more than 30 % of the LOQ and above 20 % of the highest level detected in samples.

5.2. Method for Determination of NF-180 in Sediment

5.2.1. Selectivity

NF-180 was analysed using LC-MS/MS considered as a highly selective method monitoring two specific transitions.
The evaluated transitions have been verified. Ion scan figure is shown in appendix.

5.2.2. Specificity

Two (2) control samples of sediment were analysed to investigate residue level and to check for any background interferences at the expected retention time. In addition, at least one (1) reagent blank (sample extraction procedure without matrix) was run within the validation set.

For both mass transitions, control samples showed no significant interferences (above 30 % of LOQ) at the retention time of NF-180 in sediment; therefore, the specificity of the method is sufficiently proven.

5.2.3. Matrix Effects

Matrix effect was assessed by mean response factor (RF) comparison of matrix matched standards and standards solutions. Matrix effect was calculated as follow and a correction of blank value was applied where appropriate:

$$Matrix\ Effect(\%)\quad \frac{RF_{(Matrix\ Matched\ standards)} - RF_{(Solvent\ standards)}}{RF_{(Solvent\ standards)}} \times 100$$

Table 14: Matrix effect in sediment

Analytes	Transition (m/z)	Sediment
NF-180	348.0 → 330.1 Quantification)	5.5 %
	348.0 → 180.0 Confirmation)	3.2 %

Response factor RSD was below 20%

Due to non-significant matrix effect (< ±20%) on signal, quantification using standard solutions prepared in solvent was performed.

5.2.4. Linearity

The linearity of the response was demonstrated by single determination standards calibration prepared in solvent on at least 5 concentration levels ranging from 0.005 ng/mL to 0.1 ng/mL (corresponding to 0.0125 mg/kg to 0.25 mg/kg in sediment) for analysis of NF-180. This covers the range from no more than 30 % of the LOQ and above 20 % of the highest level detected in samples.