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SYN547407 – Validation of Analytical Method GRM072.12A for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431 and SYN550738 in Soil

Final Report Amendment 1

TEST GUIDELINE(S): EPA 850.6100
EC SANCO/3029/99 rev 4
EC SANCO/825/00 rev 8.1

Deviations from the study plan

The study was performed according to the study plan dated 06 Dec 2017 and the study plan amendment no. 1 dated 07 May 2018, with no deviations. This report reflects the conduct of this study.

Abbreviations and Symbols

Abbreviation	Definition
^{o}C	Degrees Celsius or Centigrade
CAS	Chemical Abstract Services
EPA	Environmental Protection Agency (U.S.)
EC	European Commission
g	Gram
GRM	Global Residue Method
HPLC	High performance liquid chromatography
kg	Kilogram
L	Litre
LC-MS/MS	Tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
µg	Microgram
µL	Microliter
µm	Micrometre
mg	Milligram
mL	Millilitre
mm	Millimetre
min	Minute
ms	Millisecond
MS/MS	Tandem mass spectrometry
m/z	Mass to charge ratio
ng	Nanogram
OECD	Organisation for Economic Co-operation and Development
ppb	Parts per billion or micrograms per kilogram or micrograms per litre
ppm	Parts per million or milligrams per kilogram or milligrams per litre
pg	Picogram
r	Correlation coefficient
RSD	Relative Standard Deviation
V	Volt

1. EXECUTIVE SUMMARY

1.1 Study Design

Analytical Procedure

In summary, a sample of soil was extracted with acetonitrile/water (80:20 v/v) followed by centrifugation and decanting of the supernatant. The soil sample was extracted a second time with acetonitrile/ ultra pure water /acetic acid (80:20:0.1 v/v/v) followed by centrifugation and decanting of the supernatant. The supernatants were combined and then an aliquot of the combined extracts was centrifuged a further time. An aliquot was then diluted 1:1 with ultra pure water and analysed by LC-MS/MS.

Selectivity

Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix so that a high level of selectivity was demonstrated.

1.2 Results

Matrix Effects

Matrix effects on the detection of SYN547407, SYN549431, SYN549107 and SYN550738 in extracts of soil type LUFA F2.1 (batch 2817) and soil type LUFA F5M (batch 0915) were found to be insignificant (<20%). However, matrix-matched standards were used for quantification for all analytes.

Linearity

The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at six (6) concentration levels ranging from 0.015 ng/mL to 10 ng/mL. This range corresponds to equivalent sample concentrations of 0.0003 mg/kg to 0.2 mg/kg.

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

Quantification

Quantification was performed by using linear regression with additional correction for bracketing standards.

2. INTRODUCTION

2.1 Scope of the Method

Analytical method GRM072.12A is suitable for the determination of SYN547407 and its metabolites SYN549107, SYN549431 and SYN550738 in soil. The limit of quantification (LOQ) of the method has been established at 0.001 mg/kg (or 0.001 ppm, 1 ppb).

2.2 Stereospecifcity

SYN547407 is comprised of a diastereomeric pair; this method does not resolve the diastereomers or the R and S enantiomers of each diastereomeric pair and will detect and quantify all enantiomers of SYN547407 as a single chromatographic peak. SYN549107, SYN549431 and SYN550738 have one chiral centre comprised of R and S enantiomers; this method does not resolve the enantiomers of each metabolite and will detect and quantify each individual compound as a single chromatographic peak.

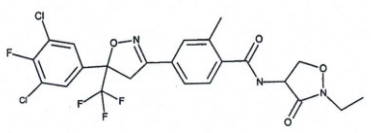
2.3 Method Summary

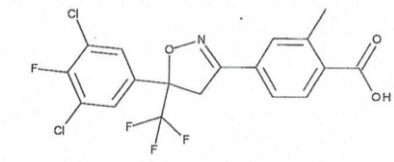
Soil samples (10 g) were extracted with acetonitrile/water (80:20 v/v) followed by centrifugation and decanting of the supernatant. The soil samples were then extracted a second time with acetonitrile/ ultra pure water /acetic acid (80:20:0.1 v/v/v) followed by centrifugation and decanting of the supernatant.. The supernatants were combined and then an aliquot of the combined extracts was centrifuged a further time. An aliquot was then diluted 1:1 with ultra pure water and analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

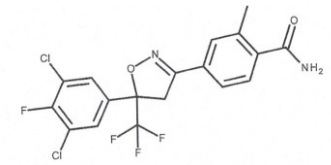
The limit of quantification of the method is 0.001 mg/kg (0.001 ppm, 1 ppb).

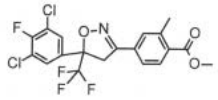
3. MATERIALS AND METHODS

3.1 Test items

Test Item 1			
Test item name	SYN547407	Batch number	AMS 1521/2
EAS Test item code	M-00009579	Appearance / colour	solid / white
Chemical name	4-(5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-N-(2-ethyl-3-oxo-1,2-oxazolidin-4-yl)-2-methylbenzamide (IUPAC)		
CAS number	not available	Purity analysed as sum of: SYN548088 SYN548089 SYN548090 SYN548091	98.4 % w/w 92.1 % w/w 1.2 % w/w 4.5 % w/w 0.6 % w/w
Chemical structure		Molecular weight	548.3 /mol
Density	not applicable	Signal word(s)	warning
Issue date of certificate	19 Sep 2016	Expiry date	31 Aug 2018
		Storage conditions	ambient (5 °C - 30 °C), dark, dry

Test Item 2			
Test item name	SYN549107	Batch number	MES 421/2
EAS Test item code	M-00014669	Appearance / colour	solid / off-white
CAS number	not available	Purity analysed	95 % w/w
Chemical structure		Molecular weight	436.2 g/mol
Density	not applicable	Signal word(s)	none
Issue date of certificate	20 Nov 2017	Expiry date	30 Nov 2019
		Storage conditions	cool (1 °C - 10 °C), dark, dry

Test Item 3			
Test item name	SYN549431	Batch number	MES 431/2
EAS Test item code	M-00014683	Appearance / colour	solid / white
Chemical name	4-(5-(3,5-dichloro-3-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-2-methylbenzamide		
CAS number	not available	Purity analysed	96 % w/w
Chemical structure		Molecular weight	435.2 g/mol
Density	not applicable	Signal word(s)	not available
Issue date of certificate	29 Sep 2017	Expiry date	30 Sep 2019
		Storage conditions	deep frozen (≤ -18 °C), dark, dry

Test Item 4			
Test item name	SYN550738	Batch number	MES 524/2
EAS Test item code	M-00014684	Appearance / colour	solid / light yellow
Chemical name	4-(5-(3,5-dichloro-3-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-2-methylbenzamide		
CAS number	not available	Purity analysed	96 % w/w
Chemical structure		Molecular weight	450.2 g/mol
Density	not applicable	Signal word(s)	none
Issue date of certificate	23 Oct 2017	Expiry date	30 Sep 2019
		Storage conditions	cool (1 °C - 10 °C), dark, dry

Specifications essential for correct identification of the test item for use under GLP are based on the certificate of analysis as provided by the sponsor. They have not been verified by the test facility and might not be generated under GLP, except where this is explicitly claimed on the certificate of analysis. Additional specifications for test item characterisation may originate from (non-GLP) sources other than the sponsor.

3.2 Apparatus

Laboratory balance (Sartorius, Kern)
Volumetric pipettes (Brand, Eppendorf)
Vortex mixer (Scientific industries)
Horizontal flatbed shaker (Bühler)
Centrifuge (Hettich, Thermo)
Sample concentrator (Stuart)
LC column Kinetex C18 (50 mm x 2.1 mm, 2.6 µm, Part number 00B-4462-AN, Phenomenex) with pre-column (KJ0-4282, Phenomenex) with 4 mm C18 cartridge (AJ0-4287, Phenomenex)
API 6500+ LC-MS/MS system (Sciex) with Agilent 1290 Infinity II

3.3 Reagents and Safety Information

Reagent	Supplier	Safety information
Acetonitrile, HPLC grade	Prolabo 83639.320	Highly flammable, poisonous by inhalation and ingestion, irritating to skin and eyes
Water, HPLC grade	Merck 1.15333	No known significant effects or critical hazards
Acetic Acid, p.a.	Prolabo 20104.298	Flammable liquid and vapour, causes severe skin burns and eye damage
Demineralised Water	Prepared at laboratory	No known significant effects or critical hazards

3.4 Preparation of Analytical Standard Solutions

3.4.1 Stock Solutions

Individual stock solutions of SYN547407, SYN549107, SYN549431 and SYN550738 were prepared by accurately weighing out the respective analytical standard into separate volumetric flasks (10 mL). The solids were dissolved in acetonitrile and diluted to mark to give stock solutions of SYN547407, SYN549107, SYN549431 and SYN550738 at concentrations of 1279 µg/mL, 1055 µg/mL, 1123 µg/mL and 1075 µg/mL, respectively. The concentrations were corrected for the purity of the test items as annotated on the CoAs.

3.4.2 Fortification Solutions

Mixed fortification solutions were prepared by diluting stock solutions of SYN547407, SYN549107, SYN549431 and SYN550738 to a final concentration of 1 µg/mL in acetonitrile and by further diluting the 1 µg/mL mixed standard solution to a final concentration of 0.1 µg/mL also with acetonitrile.

3.4.3 Preparation of Calibration Standards for LC-MS/MS

Solvent based calibration standards were prepared by dilution of a mixed standard solution of 10 µg/mL in acetonitrile with acetonitrile/water (50:50, v/v).

Matrix matched calibration standards were prepared by serial dilution of mixed standard solutions in acetonitrile/water (50:50, v/v) into a final solution of representative matrix blank extract which was generated by completing the procedure described below with untreated matrix.

Calibration standards were prepared at six (6) concentration levels ranging from 0.015 ng/mL to 10 ng/mL. This range corresponds to 0.0003 mg/kg to 0.2 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 30 % of the highest analyte concentration level detected in a sample extract (see FIGURES 113 - 144).

The fitting was performed with 1/x weighting.

4. RESULTS AND DISCUSSION

4.1 Analytical Procedure

4.1.1 Sample Preparation

Two different certified soil types were used in a homogenised state and were stored at room temperature until use.

4.1.2 Sample Fortification

At least one reagent blank and two control samples were analysed for each type of soil.

To each pre-weighed control soil sample, the appropriate amount of standard solution containing SYN547407, SYN549107, SYN549431 and SYN550738 in acetonitrile was added. Each sample was left to stand for at least five minutes after fortification to allow the spiking solution to soak into the soil before proceeding with the extraction.

4.1.3 Workup method

A summary of the method is included in flow-chart in APPENDIX 1.

a) For extraction 10 g of soil were weighed into a 250 mL polypropylene bottle; fortification solution was added at this step if required and the sample left for 5 min

b) 50 mL of acetonitrile/water (80:20 v/v) was added.

c) Samples were shaken for 1 hour using a flatbed shaker at a rate of 250 rpm.

d) Samples were centrifuged at 3500 rpm for 3 minutes.

e) The supernatant was decanted into a 100 mL volumetric flask.

f) The remaining soil was extracted with 50 mL of acetonitrile/ultra-pure water/acetic acid (80:20:0.1 v/v/v) by shaking the sample as above for 1 hour.

g) Samples were centrifuged at 3500 rpm for 3 minutes.

h) The supernatant was again decanted into the same 100 mL volumetric flask as above. The volume was adjusted to 100 mL with acetonitrile/water (80:20, v/v) and the solution was homogenised by shaking by hand.

i) An aliquot of the combined solution was transferred into a 50 mL centrifuge tube and centrifuged for 5 min at 10000 rpm.

j) 500 µL of the supernatant was diluted with 500 µL of ultra-pure water in a HPLC vial and analyzed by LC-MS/MS.

4.2 FINAL DETERMINATION

4.2.1 Instrument Description

Detector	:	Sciex QTRAP 6500+
LC System	:	Agilent Infinity II
Gas Supply	:	House Supply

4.2.2 Chromatography Conditions for SYN547407, SYN549107, SYN549431 and SYN550738

Column	:	Kinetex C18 (50 mm x 2.1 mm, 2.6 µm, 00B-4462-AN, Phenomenex) with pre-column (KJ0-4282, Phenomenex) with 4 mm C18 catridge (AJ0-4287, Phenomenex)
Column Oven Temperature	:	30 °C
Injection volume	:	30 µL
Stop Time	:	8 min
Injection protocol	:	Analyse calibration standard after 3 - 5 sample injections
Mobile phase	:	(A) Ultra-Pure Water + 0.1% Acetic Acid (B) Acetonitrile + 0.1 % Acetic Acid

Mobile Phase Composition

Time (mins)	% solvent A	% solvent B	Flow rate (mL/min)
0	60	40	0.6
1.0	60	40	0.6
6.0	5	95	0.6
7.0	5	95	0.6
7.1	60	40	0.6
8.0	60	40	0.6

Valve Switching program

Time (mins)	
0	To waste
2.7	To mass spectrometer
5.5	To waste

Under these conditions, SYN549431, SYN547407, SYN549107 and SYN550738 eluted after 3.5, 3.9, 4.3 and 5.2 minutes respectively.

4.2.3 Mass Spectrometer Conditions for SYN547407 and SYN549431

Interface	:	TurboIonSpray
Polarity	:	Positive (+)
Curtain gas (CUR)	:	30 (arbitrary units)
Temperature (TEM)	:	550 °C
Ionspray voltage	:	4500 V in positive mode; -4500 V in negative mode
Collision gas setting (CAD)	:	8 (arbitrary units)
Gas 1 (GS1)	:	30 (arbitrary units)
Gas 2 (GS2)	:	60 (arbitrary units)
Scan type	:	MRM

MRM Conditions		**SYN547407 primary transition**	**SYN547407 confirmatory transition**	**SYN549431 primary transition**	**SYN549431 confirmatory transition**
Q1 *m/z*	:	548	548	435	435
Q3 *m/z*	:	418	160	392	160
Dwell time	:	25 ms	25 ms	25 ms	25 ms
Declustering potential (DP)	:	60 V	60V	60 V	60V
Entrance potential (EP)	:	14 V	14V	14 V	14V
Collision energy (CE)	:	30 V	60 V	30 V	45 V
Collision cell exit potential (CXP)	:	15 V	15 V	15 V	15 V

4.2.4 Mass Spectrometer Conditions for SYN549107 and SYN550738

Interface	:	TurboIonSpray
Polarity	:	Period 1: 0.0 - 4.5 min: Positive/negative switching mode (+/-) Period 2: 4.5 - 8.0 min: Negative mode (-)
Curtain gas (CUR)	:	30 (arbitrary units)
Temperature (TEM)	:	550 °C
Ionspray voltage	:	4500 V
Collision gas setting (CAD)	:	8 (arbitrary units)
Gas 1 (GS1)	:	30 (arbitrary units)
Gas 2 (GS2)	:	60 (arbitrary units)
Scan type	:	MRM

MRM Conditions		**SYN549107 primary transition**	**SYN549107 confirmatory transition**	**SYN550738 primary transition**	**SYN550738 confirmatory transition**
Q1 *m/z*	:	434	434	448	448
Q3 *m/z*	:	238	354	378	69
Dwell time	:	100 ms	100 ms	100 ms	100 ms
Declustering potential (DP)	:	-60 V	-60 V	-60 V	-60 V
Entrance potential (EP)	:	-14 V	-14 V	-14 V	-14 V
Collision energy (CE)	:	-35 V	-30 V	-18 V	-60 V
Collision cell exit potential (CXP)	:	-15 V	-15 V	-15 V	-15 V

Typical chromatograms are shown in the Figures Section.

4.2.5 Confirmatory Procedures for SYN547407, SYN549107, SYN549431 and SYN550738

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

4.3 CALCULATION OF RESULTS

4.3.1 Multi Point Calibration Procedure

SYN547407, SYN549107, SYN549431 and SYN550738 residues were calculated in mg/kg for each sample as follows.

a) Standard solutions over a concentration range from 30% LOQ to at least 20% above the highest fortified level were prepared. Six (6) different concentrations within this range were prepared.

b) Injections of each sample solutions were made and the areas of the peaks corresponding to SYN547407, SYN549107, SYN549431 and SYN550738 were analysed. Calibration standard solutions were interspersed throughout the analysis, after a maximum of five injections of sample solutions.

c) Calibration curve parameters were generated using an appropriate regression package (Analyst® 1.6.3). 1/x weighting was applied to improve accuracy .

d) A regression equation to calculate the residues was generated using Analyst® 1.6.3.

e) The SYN547407, SYN549107, SYN549431 and SYN550738 residues in the sample were calculated, expressed as mg/kg, as follows

$$\text{Residue (mg/kg)} = \frac{c_1 \cdot c_{sample} \cdot V_{ex} \cdot V_{end}}{c_2 \cdot W \cdot V_{ali} \cdot 1000}$$

With:
- c_1 Nominal concentration of bracketing matrix standards (ng/mL)
- c_2 Average calculated concentration of matrix standards bracketed between samples, obtained from the matrix calibration function (ng/mL)
- c_{sample} Analysed concentration of the sample, as calculated from the matrix calibration function (ng/mL)
- V_{ex} Extraction volume (100 mL)
- V_{end} Final volume (1 mL)
- W Sample weight (10 g)
- V_{ali} Aliquot (0.5 mL)
- 1000 Conversion from ng/g to mg/kg

4.4 CONTROL AND RECOVERY SAMPLES

Control samples were analysed with each set of samples to verify that the material used to prepare the recovery samples is free from contamination. Two (2) control samples for each type of soil were analysed.

One reagent blank was analysed with each set of samples to verify that the reagents used to prepare recovery samples are free from contamination.

A total of ten (10) recovery samples for each type of soil (control samples accurately fortified with known amounts of SYN547407, SYN549107, SYN549431 and SYN550738) were analysed, where five (5) recovery samples were fortified at 0.001 mg/kg (LOQ) and five (5) were fortified at 0.01 mg/kg (10x LOQ).

Recovery efficiency was considered acceptable since the mean values were between 70% and 110% and with a relative standard deviation of <20%.

4.5 SPECIFICITY

4.5.1 Matrix

The effect of matrix on the LC-MS/MS response was assessed by comparing mean response factors of matrix-matched standards (≥ 90 % matrix amount) with solvent standards at identical concentrations.

The effects of the matrix are summarised in TABLE 23.

4.5.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found.

4.6 METHOD VALIDATION

4.6.1 Recovery Data and Repeatability

Method validation has been carried out on the procedures described in Sections 4 and 5. The method validation data are summarised in TABLE 1 - 8.

4.6.2 Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110 % with a relative standard deviation of ≤ 20% has been obtained.

The limit of quantification has been set at 0.001 mg/kg (0.001 ppm, 1 ppb).

4.6.3 Limit of Detection (LOD)

The limit of detection of the method is defined as 30 % of the LOQ
(LOD = 0.0003 mg/kg).
The LOD was determined as 0.45 pg injected on column, equivalent to 0.015 ng/mL when using a 30 µL injection volume.

4.6.4 Matrix Effects

The magnitude of the matrix effects were considered not to be significant for SYN547407, SYN549107, SYN549431 and SYN550738 in the soils tested. However, to compensate for any matrix effects matrix matched standards were routinely used.

4.6.5 Detector Linearity

Standards in the range of 30% LOQ – 200x LOQ were injected and the peak areas plotted against the nominal standard concentration for SYN547407, SYN549107, SYN549431 and SYN550738.

The linearity of the LC-MS/MS detector response for SYN547407, SYN549107, SYN549431 and SYN550738 was tested in the range from 0.45 pg to 300 pg injected on the column (equivalent to 0.015 ng/mL to 10 ng/mL when using a 30 µL injection volume). 1/x weighting was used to generate calibration curves. This range corresponds to equivalent sample concentrations of 0.0003 mg/kg to 0.2 mg/kg

4.6.6 Storage Stability

The fortification solutions prepared in acetonitrile at concenctrations of 1 µg/mL and 0.1 µg/mL were stored at 1 °C to 10 °C for 27 days in the dark, which was sufficient to cover the length of time they were used in this study. After this time, freshly prepared dilutions of the stored fortification solutions were compared to freshly prepared dilutions of freshly prepared fortification solutions by triplicate injection. One mass transition per analyte was evaluated (primary transition).

Results obtained are summarised in TABLE 9.

The calibration solution prepared in acetonitrile at a concenctration of 10 µg/mL and was stored at 1 °C to 10 °C for 27 days in the dark, which was sufficient to cover the length of time it was used in this study. After this time, freshly prepared dilutions of the stored calibration solution were compared to freshly prepared dilutions of freshly prepared calibration solutions by triplicate injection. One mass transition per analyte was evaluated (primary transition).

The mean response factor of the stored calibration and fortification solutions were within ± 20 % of the mean response factors of the freshly prepared standard and fortification solutions indicating that these solutions are stable when stored at 1 °C to 10 °C for at least 27 days in the dark.

4.6.7 Extract Stability

Following the first analysis, the final extracts fortified at the LOQ level together with two control sample extracts were stored at 1 °C to 10 °C for at least 8 days in the dark. After this period, the final extracts were re-analysed against freshly prepared calibration standards by triplicate injection. One mass transition per analyte was evaluated (primary transition).

The absolute mean recovery values of the re-analysed extracts were in the range of 70 - 110 % and within ± 20 % of the original result. Therefore, extracts are considered to be stable when stored at 1 °C to 10 °C for at least 8 days in the dark.

5. CONCLUSIONS

The used procedure has been demonstrated to be a reliable and accurate procedure for the determination of SYN547407, SYN549107, SYN549431 and SYN550738 in two types of soil (sandy loam and loamy sand according to USDA). The limit of quantification of the method is 0.001 mg/kg.

This method satisfies EC Guidance Document SANCO/3029/99 rev 4 and EC Guidance Document SANCO/825/00 rev 8.1.

TABLE 23: LC-MS/MS Matrix Effects

	Mean Matrix Effect (%)	
Analyte	**LUFA F5M**	**LUFA F2.1**
SYN547407 *m/z* 548 → 418	(-) 4	(+) 3
SYN547407 *m/z* 548→ 160	(-) 5	(+) 3
SYN549107 *m/z* 434 → 238	(+) 2	(+) 14
SYN549107 *m/z* 434 → 354	(+) 3	(+) 13
SYN549431 *m/z* 435 → 392	(-) 1	(+) 8
SYN549431 *m/z* 435 → 160	(-) 5	(+) 8
SYN550738 *m/z* 448 → 378	(-) 11	(-) 16
SYN550738 *m/z* 448 → 69	(-) 11	(-) 14

(+) matrix enhancement; (-) matrix suppression

APPENDIX 1 METHOD FLOW CHART

Weigh soil sample (10 g) into a polypropylene bottle

↓

Fortify sample as appropriate for procedural recoveries

↓

Add extraction solvent acetonitrile/water (80:20 v/v)

↓

Extract by shaking (60 minutes) on a horizontal flatbed shaker

↓

Centrifuge sample at 3500 rpm for 3 minutes and decant supernatant into volumetric flask

↓

Add extraction solvent acetonitrile/ ultra pure water /acetic acid (80:20:0.1 v/v/v) to remaining soil

↓

Extract by shaking (60 minutes) on a horizontal flatbed shaker

↓

Centrifuge sample at 3500 rpm for 3 minutes

↓

Combine extracts and fill up to 100 mL if required with acetonitrile/ultra pure water (80/20, v/v)

↓

Transfer aliquot of combined solution in 50 mL centrifuge tube

↓

Centrifuge sample at 10000 rpm for 5 minutes

↓

Dilute 500 µL of the supernatant with 500 µL of ultra-pure water in a HPLC vial

↓

Analyse by LC-MS/MS

APPENDIX 3 CHARACTERISATION OF SOIL

Soil sample	Lufa 2.1
Batch	2817
Sampling Date	13 Jul 2017
Sampling Location	Am Hanhofer Weg links, Nr. 3336/58 Germany / Rheinland-Pfalz / Dudenhofen (Country/State/Community)

Paramter	**Unit**	**Result**
pH-value (in 0.01 M $CaCl_2$)	-	5.32
pH-value (in H_2O)	-	6.21
Cation Exchange Capacity (CEC)	mmol/100g	0.7
Total Organic Carbon (TOC)	%	0.58
Organic Matter (C_{org})	%	0.99
Water Holding Capacity (WHC_{Max})	g/100g	30.1
Soil Density	g/L	1508

Particel size distribution according to USDA

Fraction	**Range**	**Soil Number**
		28 / 17 LUFA 2.1 Batch 2817
Soil Type		loamy sand
clay	< 0.002 mm	2.9
silt	0.002 – 0.050 mm	10.8
sand	0.050 – 2.00 mm	86.3

Soil sample	Lufa 5M
Batch	0915
Sampling Date	25 Feb 2015
Sampling Location	In der Speyerer Hohl, Nr. 977 Germany / Rheinland-Pfalz / Mechtersheim (Country/State/Community)

Paramter	**Unit**	**Result**
pH-value (in 0.01 M $CaCl_2$)	-	7.34
Cation Exchange Capacity (CEC)	meq*/100g	20.3
Chalk content ($CaCO_3$)	%	8.0
Organic Matter (C_{org})	%	1.11
Soil Density	g/L	1262

Particel size distribution according to USDA

Fraction	**Range**	**Soil Number**
		09 / 15 F5M 0915
Soil Type		sandy loam
clay	< 0.002 mm	10.3
silt	0.002 – 0.050 mm	32.3
sand	0.050 – 2.00 mm	57.4

*2 x mmol/100g = meq/100g