

1.0 EXECUTIVE SUMMARY

Analytical method GRM030.04A has been validated for the determination of NOA449280 (Figure 1) and its metabolites SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 (Figures 2 - 6 respectively) in soil. The limit of quantification (LOQ) of the method has been established at 0.001 mg/kg for these analytes.

A solvent blank sample was analysed, control samples were analysed in duplicate and fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.001 mg/kg) and in quintuplet at ten times the LOQ (0.010 mg/kg) for each matrix. Soils from Iowa and Nebraska were analyzed. Acceptable mean recoveries of between 70% and 110% with a relative standard deviation of <20% were found for all the primary and confirmatory transition ions on both matrices tested.

No significant suppression or enhancement of the instrument response for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 has been observed in the soil types tested using the above procedure in this laboratory. Samples should be quantified against non-matrix calibration standards where possible. Any significant matrix effects observed may be compensated for using matrix matched standards, at the discretion of the study director or by making further sample dilutions where sensitivity allows.

The linearity of the LC-MS/MS detector response for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573, was tested over the range from 2 pg to 100 pg injected on column (equivalent to 0.0002 µg/mL to 0.01 µg/mL standards when using a 10 µL injection volume) and was found to be linear. If a residue beyond the tested concentration range is expected, dilute the extract appropriately to bring it within the tested linear range prior to quantification.

Standards at 6 different concentration levels were injected and the standard concentration plotted against area counts, using Analyst 1.4.2 for both the primary and confirmatory transitions of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573.

NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 residues in final soil samples in methanol retained in vials and stored at a temperature of < 5°C were shown to be stable when stored for periods of up to 67 days, when re-analysed against a freshly prepared calibration standard.

The method has been tested on representative soil types. It can reasonably be assumed that the method can be applied to other soil types not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 residues in soil. Only commercially available laboratory equipment and reagents are required. The analysis of up to 12 samples can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification of the method is 0.001 mg/kg for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573.

The repeatability and specificity of the method have been demonstrated and GRM030.04A has been validated successfully for the determination of residues of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 residues in soil at the LOQ of 0.001 mg/kg. The method has been validated according to the EU guidelines SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7. The method validation also complies with US EPA guideline OPPTS 850.7100.

2.0 INTRODUCTION

Analytical method GRM030.04A has been developed in Syngenta laboratories for the determination of residues of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 residues in soil at the LOQ of 0.001 mg/kg using commercially available instrumentation (Ref 1). This study was conducted to validate the analytical method.

This study was conducted in accordance with guidelines SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7.

Specifically:

- a) To establish that the method will produce recovery values which are within an acceptable range (i.e. mean recoveries between 70% and 110%, with a relative standard deviation within a run of $\leq 20\%$), for each fortification level and overall.
- b) To establish that the limit of quantification (LOQ) of the analytical method is 0.001 mg/kg for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573.
- c) To establish that residues of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in control samples are not present at levels above 30% of the LOQ.
- d) To investigate the relationship between instrument response and analyte concentration over concentration ranges typical of those for which the method will be used.
- e) To assess suppression or enhancement of instrument response to analyte in the presence of matrix.
- f) To assess the stability of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 stored at $<5^{\circ}\text{C}$ in final extracts from soil.
- g) The limit of detection (LOD) will be assessed and reported.
- h) This study has been designed to comply with SANCO/3029/99 rev.4 and SANCO/825/00 rev.7 guidelines. The method validation also complies with US EPA guideline OPPTS 850.7100.

3.0 MATERIALS AND METHODS

3.1 Test and Reference substances

Figure 1

Compound Code : NOA449280
Number
CAS Number : 352010-68-5
IUPAC Name : 4-Hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-trifluoromethyl-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one
Molecular Formula : C₁₉H₂₀F₃NO₅
Molecular Weight : 399.4

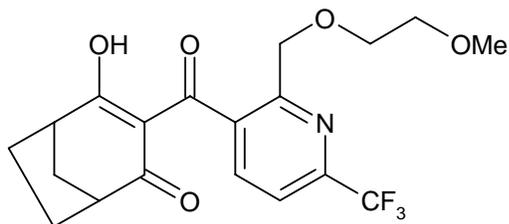


Figure 2

Compound Code : SYN503780
Number
Alternate Compound : CSAA794148
Code Number
CAS Number : 380355-55-5
IUPAC Name : 2-(2-Methoxy-ethoxymethyl)-6-trifluoromethyl-nicotinic acid
Molecular Formula : C₁₁H₁₂F₃NO₄
Molecular Weight : 279.2

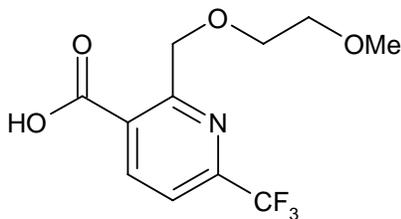
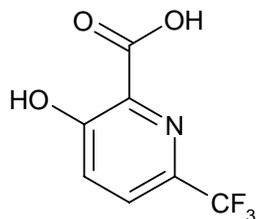


Figure 3

Compound Code Number : CSCD656832
Alternative Compound Code Number : SYN545680
CAS Number : Not listed
IUPAC Name : 3-Hydroxy-6-trifluoromethyl-pyridine-2-carboxylic acid
Molecular Formula : $C_7H_4F_3NO_3$
Molecular Weight : 207.1

**Figure 4**

Compound Code Number : CSCD642512
Alternative Compound Code Number : SYN545859
CAS Number : Not listed
IUPAC Name : 4-Hydroxy-3-[2-(2-methoxy-methyl-carboxylic acid)-6-trifluoromethyl-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one
Molecular Formula : $C_{18}H_{16}F_3NO_6$
Molecular Weight : 399.3

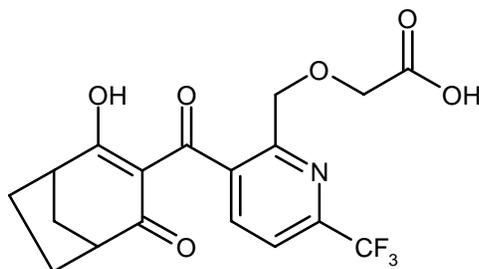


Figure 5

Compound Code Number : CSCC163768
Alternative Compound Code Number : SYN504810
CAS Number : 90376-94-6
IUPAC Name : 2,3-dicarboxylicacid-6-trifluoromethylpyridine
Molecular Formula : $C_8H_4F_3NO_4$
Molecular Weight : 235.1

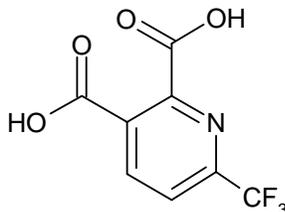
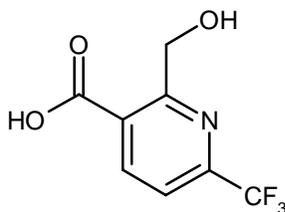


Figure 6

Compound Code Number : NOA451778
Alternative Compound Code Number : CSAA806573
CAS Number : Not listed
IUPAC Name : 2-(Hydroxymethyl)-6-trifluoromethyl-pyridine-3-carboxylic acid
Molecular Formula : $C_8H_6F_3NO_3$
Molecular Weight : 221.1



3.2 Test system

The validation study was carried out using prepared control soil samples supplied from Syngenta Crop Protection from Iowa and Nebraska. The characteristics of the soils used are presented in Table 1.

3.3 Preparation and stability of analytical standard solutions

The standard solutions were prepared as follows.

3.3.1 Stock Standard Preparation

Individual ~ 1000 µg/mL stock solutions for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 were prepared as follows:

Appropriate amounts of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 analytical standards were transferred into separate “Class A” volumetric flasks (10 mL). NOA449280, SYN503780, CSCD656832, CSCD642512 were diluted to the mark with acetonitrile to give a ~1000 µg/mL stock concentration. CSAA806573 and CSCC163768 were diluted to the mark with methanol to give a ~1000 µg/mL stock concentration. Stock standards were stored in a freezer at <-10°C for no longer than one year.

3.3.2 Fortification Standard Preparation

Sample fortification solutions were prepared in methanol from the primary stock solution in “Class A” volumetric flasks. The following mixed solutions were prepared by serial dilution: 100 µg/mL, 1.0 µg/mL and 0.1 µg/mL. Fortification standards were stored in a freezer at <-10°C for no longer than 7 months.

3.3.3 Calibration Standard Preparation

Calibration standards diluted with 10:90 methanol:water over an appropriate range were prepared for analytical determination by LC-MS/MS. To prepare, for example, an LOQ equivalent non-matrix calibration standard (0.0004 µg/mL) of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573, transfer 80 µL of 5.00 ng/mL mixed NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 to an autosampler vial. Add 920 µL of 10:90 methanol:water.

The calibration standard solutions were stored at <5°C for no longer than one week.

3.4 Fortification levels

Recovery of the analyte through the analytical procedure was assessed by fortifying 5 aliquots of soil with NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 at the LOQ of 0.001 mg/kg and 5 aliquots of soil with NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 at ten times the LOQ, i.e. 0.010 mg/kg. In addition, two control samples and one solvent blank were analysed with each sample batch. Fortification levels are summarised in Table 2.

3.5 Analytical procedures

3.5.1 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise.

The limit of detection for this procedure in the soil types tested is estimated at 0.5 µg/kg for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573, for both the primary and confirmatory transitions.

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 $\leq 20\%$ has been obtained. Generally, for accurate quantification, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantification has been set at 0.001 mg/kg for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573.

3.5.2 Sample analysis

Samples were analysed as follows:

10 g soil samples were heated at reflux in 50:50 v/v acetonitrile:1 M NH₄OH for 1 hour. After cooling to room temperature, extracts were centrifuged and then aliquots were removed for clean-up and concentration with a solid phase extraction (SPE) procedure using Chromabond HR-P (styrene/ divinylbenzene) cartridges. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). Inconsistent recoveries were observed with the various soil types. Therefore, the following modifications were made to the reference method:

1. The extract was not concentrated to remove acetonitrile prior to SPE.
2. The extract was not diluted in water and the pH was not adjusted to <2 prior to SPE.
3. Did not use Strata-X SPE. This cleanup was not necessary and was replaced with Chromabond HR-P cartridges.

The percentage recovery obtained for each sample was calculated and these results were used to assess the relative standard deviation and limit of quantification of the analytical method (see Tables 3-14).

3.5.3 Detector linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of the detector.

In this laboratory the linearity of the LC-MS/MS detector response for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573, was tested over the range from 2 pg to 100 pg injected on column (equivalent to 0.0002 µg/mL to 0.01 µg/mL standards when using a 10 µL injection volume) and was found to be linear. If a residue beyond the tested concentration range is expected, dilute the extract appropriately to bring it within the tested linear range prior to quantification.

Standards at 6 different concentration levels were injected and the standard concentration plotted against area counts, using Analyst 1.4.2 for both the primary and confirmatory transitions of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573.

Detector linearity graphs are presented in Tables 21-26.

3.5.4 Storage stability of sample extracts

NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 residues in final soil samples in methanol retained in vials and stored at a temperature of < 5°C were shown to be stable when stored for periods of up to 67 days, when re-analysed against a freshly prepared calibration standard.

Storage stability results are summarised in Tables 15-20.

3.5.5 Matrix effects

No significant suppression or enhancement of the instrument response for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 has been observed in the soil types tested using the above procedure in this laboratory. Samples should be quantified against non-matrix calibration standards where possible. Any significant matrix effects observed may be compensated for using matrix matched standards, at the discretion of the study director or by making further sample dilutions where sensitivity allows. Full details are presented in Table 27.