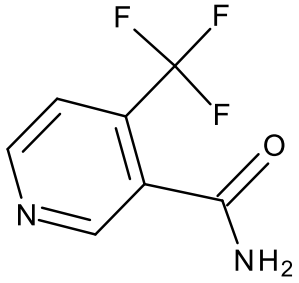
# 4-(TRIFLUOROMETHYL)-NICOTINAMIDE

**DRAFT**




| | |
|---|---|
| *ISO Common Name* | 4-(trifluoromethyl)-nicotinamide |
| *Chemical Name* | 4-(trifluoromethyl)pyridine-3-carboxamide |
| *Empirical formula* | $C_7H_5F_3N_2O$ |
| *RMM* | 190.1 |
| *m.p.* | not available |
| *CAS Number* | 158062-71-6 |

**1 Sampling.** Take at least 300 mg.

## 2 Identity test

**2.1 HPLC.** Use the HPLC method below. The relative retention time of 4-(trifluoromethyl)-nicotinamide in the sample solution should not deviate by more than 2% from that of the calibration solution. The UV spectrum measured from this peak should match that obtained from the calibration substance.
(Fig 1)

## 3 4-(Trifluoromethyl)-nicotinamide

OUTLINE OF METHOD

4-(trifluoromethyl)-nicotinamide is determined by reversed phase high performance liquid chromatography using UV detection at 265 nm and external standardization.

REAGENTS

*4-(trifluoromethyl)-nicotinamide* reference standard with known content.

*Acetonitrile* HPLC grade.

*Deionized water*.

*Phosphoric acid (85%).*

acetonitrile / 0.5% v/v phosphoric acid in water (50/50 v/v)

**Calibration solution.** Weigh in duplicate (to the nearest 0.1 mg) 90 – 110 mg (*s* mg) of 4-(trifluoromethyl)-nicotinamide reference standard into separate 100 ml volumetric flasks. Dissolve with 90 ml acetonitrile and treat for approx. 5 minutes in an ultrasonic bath or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. Dilute 10.0 ml of this solution into a 50 ml volumetric flask and make up to volume with acetonitrile / 0.5% v/v phosphoric acid in water (50/50 v/v) at ambient temperature. If necessary, clarify a portion of the solution using a PTFE 0.45 µm filter (solutions $C_A$ and $C_B$).

APPARATUS

*High performance liquid chromatograph* equipped with an ultraviolet spectrophotometric detector and an injecting system capable of injecting 5 µl.

*Column* stainless steel, 150 x 4.6 mm (i.d.), packed with Kinetex Polar C18, 2.6 µm, or equivalent material with the same selectivity.

*Ultrasonic bath*

*Dispensable PTFE filters,* solvent compatible, 25 mm, porosity 0.45 µm.

*Electronic integrator* or *data system*

PROCEDURE

**(a) Liquid chromatographic conditions (typical):**

| | |
|---|---|
| *Column* | stainless steel, 150 x 4.6 mm (i.d.), packed with Kinetex Polar C18, 2.6 µm, or equivalent material with the same selectivity. |
| *Column temperature* | 30 °C |
| *Flow rate* | 1.0 ml/min |
| *Detector wavelength* | 265 nm |
| *Injection volume* | 5 µl |
| *Run time* | 13 min |
| *Retention time* | approximately 3.5 min |

*Gradient*

| time (min) | % 0.5% v/v phosphoric acid in water | % acetonitrile |
|---|---|---|
| 0 | 95 | 5 |
| 8 | 10 | 90 |
| 10 | 10 | 90 |
| 10.1 | 95 | 5 |
| 13 | 95 | 5 |

**(b) System equilibration.** Prepare two calibration solutions. Inject 5 µl portions of solution $C_A$ until the response factors obtained for two consecutive injections differ by less than 1 %. Then inject a 5 µl portion of the solution $C_B$. The response factor for this solution should not deviate by more than 1 % from that of solution $C_A$, otherwise prepare new calibration solutions.

**(c) Sample preparation**. Prepare solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) 90 – 110 mg (w mg) of the sample into a 100 ml volumetric flask. Dissolve with 90 ml acetonitrile and treat for approx. 5 minutes in an ultrasonic bath or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. Dilute 10.0 ml of this solution into a 50 ml volumetric flask and make up to volume with acetonitrile / 0.5% v/v phosphoric acid in water (50/50 v/v) at ambient temperature. If necessary, clarify a portion of the solution using a PTFE 0.45 µm filter (solutions $S_A$ and $S_B$).

**(d) Determination.** If not otherwise requested inject in duplicate 5 µl portions of each sample solution, bracketing them with duplicate injections of the calibration solution as follows: calibration solution $C_A$, calibration solution $C_B$, calibration solution $C_A$, sample solution $S1_A$, sample solution $S1_B$, calibration solution $C_A$, sample solution $S2_A$, sample solution $S2_B$, calibration solution $C_A$, and so on for further samples. Measure the relevant peak areas.

**(e) Calculation**. Calculate the mean value of each pair of calibration response factors, bracketing the two injections of a sample, and use this value for calculating the 4-(trifluoromethyl)-nicotinamide contents of the bracketed sample injections.

$$f = \frac{s \times P}{H_s}$$

$$\text{Content of 4-(trifluoromethyl)-nicotinamide} = \frac{f \times H_w}{w} \text{ g/kg}$$

where:

$f$ = mean response factor

$H_s$ = peak area of 4-(trifluoromethyl)-nicotinamide in the calibration solution

$H_w$ = peak area of 4-(trifluoromethyl)-nicotinamide in the sample solution

$s$ = mass of 4-(trifluoromethyl)-nicotinamide reference standard in the calibration solution (mg)

$w$ = mass of sample taken (mg)

$P$ = purity of 4-(trifluoromethyl)-nicotinamide reference standard (g/kg)

**Repeatability r = to be determined**

**Reproducibility R = to be determined**

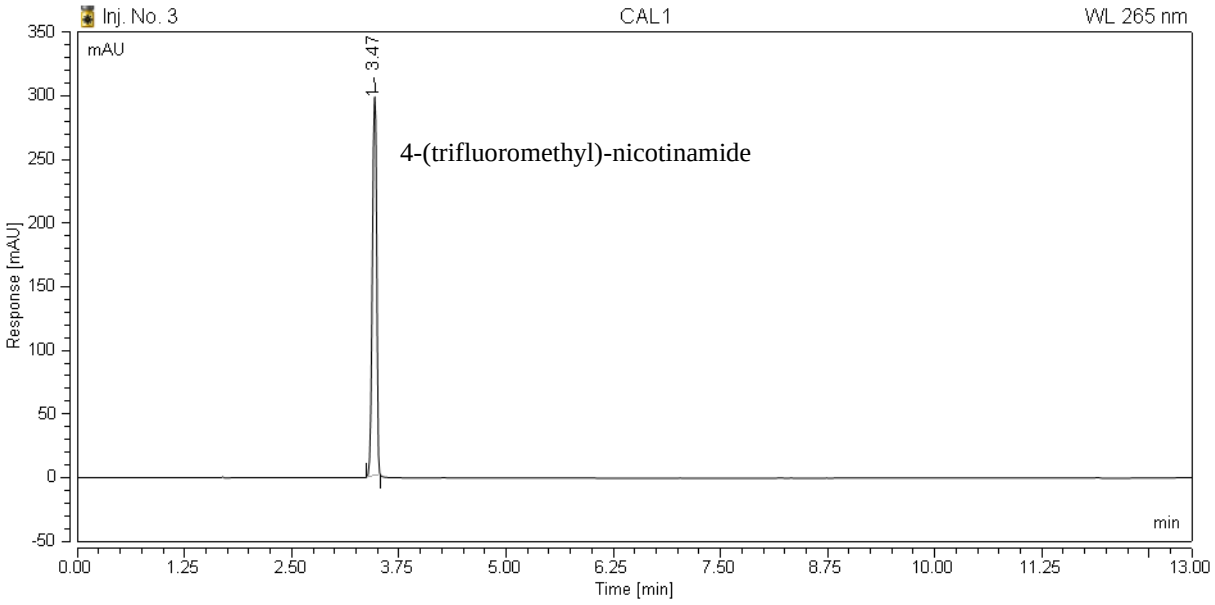



**Fig 1** Typical chromatogram of 4-(trifluoromethyl)-nicotinamide TC