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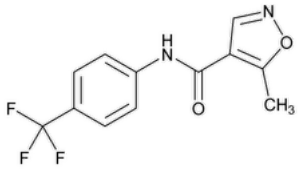
Leflunomide



$C_{12}H_9F_3N_2O_2$ 270.21

4-Isoxazolecarboxamide, 5-methyl-*N*-[4-(trifluoromethyl) phenyl]-;

α,α,α-Trifluoro-5-methyl-4-isoxazolecarboxy-*p*-toluidide CAS RN®: 75706-12-6; UNII: G162GK9U4W.

DEFINITION

Leflunomide contains NLT 98.0% and NMT 102.0% of $C_{12}H_9F_3N_2O_2$, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**▲ (CN 1-May-2020)

Sample: Dry the substance for 10 min at 130°.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile, triethylamine, and water (70:1:130). Adjust with phosphoric acid to a pH of 4.

Standard solution: 0.5 mg/mL of USP Leflunomide RS in acetonitrile and *Mobile phase* (1:9). [Note—First dissolve in acetonitrile. Protect solutions from light.]

System suitability solution: 0.5 mg/mL of USP Leflunomide RS, 0.15 mg/mL of USP Leflunomide Related Compound B RS, and 0.05 mg/mL of USP Leflunomide Related Compound C RS in *Mobile phase*. [Note—Dissolve the Reference Standards in acetonitrile, and dilute with *Mobile phase*.]

Sample solution: 0.5 mg/mL of Leflunomide in acetonitrile and *Mobile phase* (1:9). [Note—First dissolve in acetonitrile. Protect solutions from light.]

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 12.5-cm; packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for leflunomide related compound B and leflunomide related compound C are 0.2 and 0.9, respectively.]

Suitability requirements

Resolution: NLT 1.0 between the leflunomide and leflunomide related compound C peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{12}H_9F_3N_2O_2$ in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **Residue on Ignition ⟨281⟩:** NMT 0.1%

Change to read:

Organic Impurities

- **Procedure 1: Limit of Leflunomide Related Compound A**

Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: 0.125 mg/mL of USP Leflunomide Related Compound A RS, in acetonitrile and *Mobile phase* (1:19)

Standard solution: 0.5 µg/mL of USP Leflunomide Related Compound A RS, from the *Standard stock solution* in *Mobile phase*

Sample solution: 2.5 mg/mL of Leflunomide, in acetonitrile and *Mobile phase* (1:9)

Injection size: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of leflunomide related compound A in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of leflunomide related compound A from the *Sample solution*

r_S = peak area of leflunomide related compound A from the *Standard solution*

C_S = concentration of USP Leflunomide Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.02 %

- **Procedure 2**

Mobile phase, Sample solution, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

▲**Standard stock solution:** Proceed as directed in the *Standard solution* in the *Assay*.

Standard solution: 0.5 µg/mL of USP Leflunomide RS from the *Standard stock solution* in *Mobile phase*▲ (ERR 1-Feb-2019)

Sensitivity solution: 0.25 µg/mL of Leflunomide, from the *Standard solution* in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Resolution: NLT 1.0 between leflunomide and leflunomide related compound C

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Disregard any peak with an area less than the leflunomide peak from the *Sensitivity solution*. Continue the elution for two times the retention time of the leflunomide peak.]

Calculate the percentage of each related compound and any unknown impurity (see *Impurity Table 1*) in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for each impurity from the *Sample solution*

r_S = peak area of leflunomide from the *Standard solution*

C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)

C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methylisoxazole-carboxylic acid	0.05	1.0	0.1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Leflunomide related compound B	0.22	1.0	0.3
N-(2'-Trifluoromethyl phenyl)-5-methylisoxazole-4-Carboxamide	0.29	1.0	0.1
2-Cyano-acetic acid-(4'-trifluoromethyl)-anilide	0.36	1.0	0.1
Leflunomide related compound C	0.94	1.0	0.1
Any other individual impurity	—	—	0.1
Total impurities, excluding leflunomide related compound B and leflunomide related compound C	—	—	0.2
Total impurities	—	—	0.4

SPECIFIC TESTS

- **Melting Range or Temperature ⟨741⟩:** 164°–168°
- **Loss on Drying ⟨731⟩:** Dry a sample in a vacuum over diphosphorus pentoxide at 60° for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in a well-closed container. Store at a temperature not exceeding 30°.
- **USP Reference Standards ⟨11⟩**

  USP Leflunomide RS
  USP Leflunomide Related Compound A RS
  USP Leflunomide Related Compound B RS
  USP Leflunomide Related Compound C RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
LEFLUNOMIDE	Documentary Standards Support	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

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