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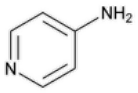
Dalfampridine



$C_5H_6N_2$ 94.11

4-Pyridinamine;

4-Aminopyridine CAS RN®: 504-24-5.

DEFINITION

Dalfampridine contains NLT 98.0% and NMT 102.0% of dalfampridine ($C_5H_6N_2$).

IDENTIFICATION

Change to read:

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

• **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**

Solution A: 3.03 g/L of sodium 1-heptanesulfonate, 1.36 g/L of monobasic potassium phosphate, and 1.15 g/L of phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
5.0	90	10
15.0	80	20
20.0	80	20
20.1	90	10
25.0	90	10

Diluent: *Solution A* and *Solution B* (90:10)

System suitability stock solution: 0.04 mg/mL of USP Dalfampridine Related Compound A RS prepared as follows. Transfer a suitable quantity of USP Dalfampridine Related Compound A RS to an appropriate volumetric flask and add 20% of the total flask volume of *Diluent*. Sonicate for about 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Use within 24 h.

System suitability solution: 0.0002 mg/mL of USP Dalfampridine Related Compound A RS from *System suitability stock solution* and 0.2 mg/mL of USP Dalfampridine RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Dalfampridine RS to an appropriate volumetric flask, add an appropriate volume of *System suitability stock solution*, and add 25% of the total flask volume of *Diluent*. Sonicate

for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter, discard NLT the first 2 mL, and use the filtrate.

Standard solution: 0.2 mg/mL of USP Dalfampridine RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Dalfampridine RS to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Sample solution: 0.2 mg/mL of Dalfampridine in *Diluent* prepared as follows. Transfer a suitable quantity of Dalfampridine to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 10.0-cm; 3.5-µm packing L1

Column temperature: 30°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between dalfampridine and dalfampridine related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dalfampridine ($C_5H_6N_2$) in the portion of Dalfampridine 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 Dalfampridine RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **Residue on Ignition ⟨281⟩**

Ignition temperature: 800°

Acceptance criteria: NMT 0.3%

• **Limit of Dalfampridine Related Compound B and Dalfampridine Related Compound C**

[Note—This test should be conducted if dalfampridine related compound B and dalfampridine related compound C are possible from the manufacturing process.]

Solution A, Solution B, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.

Standard solution: 2 µg/mL each of USP Dalfampridine Related Compound B RS and USP Dalfampridine Related Compound C RS in *Diluent*

Sensitivity solution: 0.1 µg/mL each of USP Dalfampridine Related Compound B RS and USP Dalfampridine Related Compound C RS from *Standard solution* in *Diluent*

Sample solution: 2000 µg/mL of Dalfampridine in *Diluent* prepared as follows. Transfer a suitable quantity of Dalfampridine to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume.

Chromatographic system: Proceed as directed in the *Assay*, except use an *Injection volume* of 30 µL.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Relative standard deviation: NMT 10% each for dalfampridine related compound B and dalfampridine related compound C, *Standard solution*

Signal-to-noise ratio: NLT 10 each for dalfampridine related compound B and dalfampridine related compound C, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dalfampridine related compound B and dalfampridine related compound C in the portion of Dalfampridine taken:

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

r_U = peak response of dalfampridine related compound B or dalfampridine related compound C from the *Sample solution*

r_S = peak response of dalfampridine related compound B or dalfampridine related compound C from the *Standard solution*

C_S = concentration of USP Dalfampridine Related Compound B RS or USP Dalfampridine Related Compound C RS in the *Standard solution* (µg/mL)

C_U = concentration of Dalfampridine in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.0075% each for dalfampridine related compound B and dalfampridine related compound C

- **Organic Impurities**

Solution A, Solution B, Mobile phase, Diluent, and **System suitability solution:** Prepare as directed in the *Assay.*

Standard solution: 2 µg/mL of USP Dalfampridine RS in *Diluent*

Sensitivity solution: 0.1 µg/mL of USP Dalfampridine RS from *Standard solution* in *Diluent*

Sample solution: 200 µg/mL of Dalfampridine in *Diluent* prepared as follows. Transfer a suitable quantity of Dalfampridine to an appropriate volumetric flask and add 25% of the total flask volume of *Diluent*. Sonicate for NLT 5 min. Allow to cool to room temperature and dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

Chromatographic system: Proceed as directed in the *Assay*, except use a *Detector* wavelength of 265 nm for isonicotinamide.

System suitability

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

[Note—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between dalfampridine and dalfampridine related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for dalfampridine, *System suitability solution*

Relative standard deviation: NMT 5.0% for dalfampridine, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isonicotinamide in the portion of Dalfampridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of isonicotinamide at 265 nm from the *Sample solution*

r_S = peak response of dalfampridine at 275 nm from the *Standard solution*

C_S = concentration of USP Dalfampridine RS in the *Standard solution* (µg/mL)

C_U = concentration of Dalfampridine in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Calculate the percentage of dalfampridine related compound A and any other unspecified impurity in the portion of Dalfampridine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of dalfampridine related compound A or any unspecified impurity at 275 nm from the *Sample solution*

r_S = peak response of dalfampridine at 275 nm from the *Standard solution*

C_S = concentration of USP Dalfampridine RS in the *Standard solution* (µg/mL)

C_U = concentration of Dalfampridine in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isonicotinamide	0.64	0.45	0.15
Dalfampridine	1.0	—	—
Dalfampridine related compound A	1.2	1.6	0.10
Dalfampridine related compound B[a]	2.4	—	—
Dalfampridine related compound C[a]	6.4	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities[b]	—	—	0.50

[a] This impurity is quantified using the *Limit of Dalfampridine Related Compound B and Dalfampridine Related Compound C* test.

[b] The sum of all impurities from the test for *Organic Impurities* and the *Limit of Dalfampridine Related Compound B and Dalfampridine Related Compound C* test.

SPECIFIC TESTS

• **Water Determination ⟨921⟩, *Method I, Method Ia*:** NMT 0.3%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards ⟨11⟩**

USP Dalfampridine RS

USP Dalfampridine Related Compound A RS

4-Aminopyridine 1-oxide.

$C_5H_6N_2O$ 110.11

USP Dalfampridine Related Compound B RS

3,5-Dibromopyridin-4-amine.

$C_5H_4Br_2N_2$ 251.91

USP Dalfampridine Related Compound C RS

1,3-Di(pyridin-4-yl)urea.

$C_{11}H_{10}N_4O$ 214.23

Topic/Question	Contact	Expert Committee
DALFAMPRIDINE	Documentary Standards Support	SM42020 Small Molecules 4

Chromatographic Database Information: Chromatographic Database

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