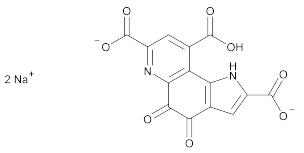
Pyrroloquinoline Quinone Disodium



$C_{14}H_4N_2Na_2O_8$ 374.17

$C_{14}H_4N_2Na_2O_8 \cdot H_2O$ 392.19

$C_{14}H_4N_2Na_2O_8 \cdot 2\ H_2O$ 410.20

$C_{14}H_4N_2Na_2O_8 \cdot 3\ H_2O$ 428.22

1*H*-Pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, 4,5-dihydro-4,5-dioxo-, sodium salt (1:2);
Sodium 9-carboxy-4,5-dioxo-4,5-dihydro-1*H*-pyrrolo[2,3-*f*]quinoline-2,7-dicarboxylate CAS RN®: 122628-50-6.
1*H*-Pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, 4,5-dihydro-4,5-dioxo-, sodium salt (1:2) trihydrate CAS RN®: 1519046-70-8.

Change to read:

DEFINITION

Pyrroloquinoline Quinone Disodium ▲▲ (USP 1-Dec-2021) contains NLT 98.0% and NMT 102.0% of pyrroloquinoline quinone disodium salt ($C_{14}H_4N_2Na_2O_8$), calculated on the anhydrous basis. ▲It may contain up to three molecules of water of hydration.▲ (USP 1-Dec-2021) Pyrroloquinoline quinone is also known as PQQ in the dietary supplements industry.

IDENTIFICATION

Change to read:

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ▲The retention time of the sodium peak of the *Sample solution* corresponds to that of the *Standard solution* at 24 µg/mL, as obtained in the test for *Content of Sodium*.▲ (USP 1-Dec-2021)

Add the following:

▲• **B.**

[NOTE—The requirements for *Identification B* may be met by following either *Procedure 1* or *Procedure 2*.]

Procedure 1: Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K

[NOTE—If the IR spectra of the sample and USP Pyrroloquinoline Quinone Disodium RS do not match (different hydration forms), separately dissolve about 3 mg each from the sample and from the USP Pyrroloquinoline Quinone Disodium RS in 0.6 mL of water. Add 0.2 mL of dehydrated alcohol, and mix well. Let the solvent evaporate from both solutions at NMT 50°. Once the solvent is evaporated, dry the sample under vacuum at room temperature.]

Procedure 2: ^{1}H NMR Spectrum

Diluent: Deuterium oxide containing 0.05% deuterated sodium-3-trimethylsilylpropionate (TMSP-d_4) NMR shift reference

Standard solution: Dissolve 2 mg of USP Pyrroloquinoline Quinone Disodium RS in 0.6 mL of *Diluent*.

Sample solution: Dissolve 2 mg of pyrroloquinoline quinone disodium in 0.6 mL of *Diluent*.

Instrumental conditions

(See *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩.)

Acquisition parameters

Mode: Fourier transform proton (^{1}H) NMR, pulsed

Frequency: NLT 300 MHz for ^{1}H

Pulse sequence: Single pulse

Pulse angle: 30°

Sweep width: 15 ppm (–2.0 to 13.0 ppm)

Acquired data points: NLT 32K (for 300 MHz)

Acquisition temperature: 22°–25°

Acquisition time: NLT 2.5 s

Relaxation delay: NLT 10 s

Number of scans: Adjust to meet the requirements for *System suitability*.

Dummy scans: 2

Processing parameters

Processed spectrum data points: NLT 64K (for 300 MHz)

Window multiplication: Exponential

Line broadening factor: 1.0 Hz

System suitability

Sample: *Standard solution*

Suitability requirements

Signal-to-noise ratio (S/N): The number of transients should be adjusted until S/N for each signal of pyrroloquinoline quinone from the *Standard solution* is NLT 100:1 (see *Table 1*).

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer the *Standard solution* and the *Sample solution* into separate NMR tubes of 5 mm in diameter. Acquire free induction decay (FID) with or without water suppression. Chemical shift for the NMR reference (TMSP-d_4 methyl signal) should be set to 0.00 ppm for all *Samples*. Immediately acquire the ^{1}H NMR spectrum for the *Standard solution* and *Sample solution* using the same conditions. Phase the spectrum and correct the baseline prior to signal identification. The ^{1}H NMR spectrum of the *Standard solution* should display two sets of ^{1}H signals, corresponding to the 4,5-*ortho*-quinone and 5,5-geminal diol water adduct forms of pyrroloquinoline quinone moiety, as shown in *Table 1*.

Table 1

Chemical shift values, δ_H (ppm)	Signal multiplicity	Signal assignment
7.19	singlet	C(3)H (pyrrole)[a]
7.22	singlet	C(3)H (pyrrole)[b]
8.20	singlet	C(8)H (pyridine)[a]
8.29	singlet	C(8)H (pyridine)[b]

[a] Corresponds to the 4,5-*ortho*-quinone form.
[b] Corresponds to the 5,5-geminal diol water adduct form.

Acceptance criteria: The chemical shift values of signals from the *Sample solution* match those obtained from the *Standard solution* and do not differ by more than ±0.1 ppm. The ratio between the sum of integrals for the C(8)H to the sum of integrals for the C(3)H resonances is 1.0 ± 0.1.▲ (USP 1-Dec-2021)

ASSAY

Change to read:

- **PROCEDURE**

Buffer solution: Prepare a solution containing 10 mM dibasic potassium phosphate and 15 mM

tetrabutylammonium bromide in water. Adjust with phosphoric acid to a pH of 7.4.
Mobile phase: Acetonitrile and *Buffer solution* (28:72)
Diluent: Acetonitrile and water (1:3)
Glycine standard solution: 0.1 mg/mL of USP Glycine RS in water
Standard solution: 0.1 mg/mL of USP Pyrroloquinoline Quinone Disodium RS in *Diluent*
System suitability solution: Transfer 1 mL of the *Standard solution* and 0.1 mL of the *Glycine standard solution* to an HPLC vial, cap the vial, and heat at 60° in a water bath for 30 min. Cool in an ice bath to room temperature and analyze immediately.
Sample solution: 0.1 mg/mL of Pyrroloquinoline Quinone Disodium in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 259 nm
Column: 4.6-mm × 15-cm; 5-µm, endcapped, packing L1 with 17% carbon load
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 20 µL
▲**Run time:** NLT 3 times the retention time of pyrroloquinoline quinone▲ (USP 1-Dec-2021)
System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The *System suitability solution* should exhibit two major peaks for pyrroloquinoline quinone and imidazolopyrroloquinoline. The relative retention times for the two peaks are 1.00 and 1.15, respectively.]
Suitability requirements
Resolution: NLT 2.5 between pyrroloquinoline quinone and imidazolopyrroloquinoline, *System suitability solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of pyrroloquinoline quinone disodium ($C_{14}H_4N_2Na_2O_8$) in the portion of Pyrroloquinoline Quinone Disodium taken:

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

r_U = peak response of pyrroloquinoline quinone from the *Sample solution*
r_S = peak response of pyrroloquinoline quinone from the *Standard solution*
C_S = concentration of USP Pyrroloquinoline Quinone Disodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Pyrroloquinoline Quinone Disodium in the *Sample solution* (mg/mL)

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

OTHER COMPONENTS

• **CONTENT OF SODIUM**
Mobile phase: 20 mM methanesulfonic acid in water
Standard solutions: Prepare solutions containing 4, 8, 12, 16, 20, and 24 µg/mL of sodium in water, from commercially available sodium standard solution for ion chromatography.
Sample solution: 0.2 mg/mL of Pyrroloquinoline Quinone Disodium in water
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: Ion chromatography
Detector: Conductivity
Columns
Guard: 4-mm × 5-cm; 7.0- to 9.0-µm packing L106
Analytical: 4-mm × 25-cm; 7.0- to 9.0-µm packing L106
Cation suppressor: Use a self-regenerating cation suppressor. Set the current to 70 milliamperes or according to manufacturer's recommendations to achieve optimal signal-to-noise ratio.
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 25 µL
Run time: NLT 3 times the retention time of sodium
System suitability
Sample: *Standard solution* (16 µg/mL)
Suitability requirements
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Correlation coefficient: NLT 0.99 for the regression line as determined in *Analysis*
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the sodium peak responses against the sodium concentrations, in mg/mL, in the six *Standard solutions*, and establish a calibration curve by least-squares regression. Using the sodium peak response from the *Sample solution*, calculate the concentration, *C*, in mg/mL, of sodium in the *Sample solution*.
Calculate the percentage of sodium in the portion of Pyrroloquinoline Quinone Disodium taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of sodium in the *Sample solution* (mg/mL), determined from the calibration curve
C_U = concentration of Pyrroloquinoline Quinone Disodium in the *Sample solution* (mg/mL)

Acceptance criteria: 10.5%–12.9% on the anhydrous basis

IMPURITIES

• **ORGANIC IMPURITIES**
Buffer solution, Mobile phase, Diluent, Glycine standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Standard solution: 0.1 µg/mL of USP Pyrroloquinoline Quinone Disodium RS in *Diluent*
Analysis
Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each individual impurity in the portion of Pyrroloquinoline Quinone Disodium taken:

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

r_U = peak response of any individual impurity from the *Sample solution*
r_S = peak response of pyrroloquinoline quinone from the *Standard solution*
C_S = concentration of USP Pyrroloquinoline Quinone Disodium RS in the *Standard solution* (mg/mL)
C_U = concentration of Pyrroloquinoline Quinone Disodium in the *Sample solution* (mg/mL)

Acceptance criteria
Any individual impurity: NMT 0.1%
Total impurities: NMT 1.0%

SPECIFIC TESTS

- **Water Determination** ⟨921⟩**,** *Method I, Method Ia***:** NMT 13.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

- **USP Reference Standards** ⟨11⟩
  USP Glycine RS
  USP Pyrroloquinoline Quinone Disodium RS