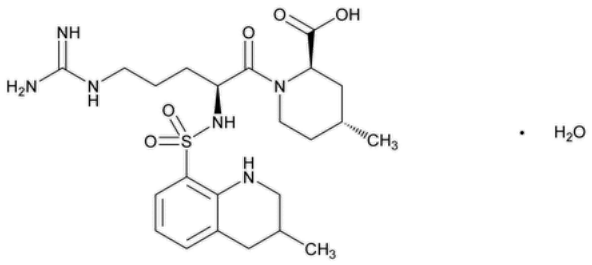
Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-May-2020
Document Type: USP Monographs
DocId: GUID-7E80D9CB-CA84-4C40-ACD4-C971E28C02A2_6_en-US
DOI: https://doi.org/10.31003/USPNF_M5830_06_01
DOI Ref: sws9f

Change to read:

Argatroban

▲



▲ (ERR 1-Mar-2019)

$C_{23}H_{36}N_6O_5S \cdot H_2O$ 526.65

2-Piperidinecarboxylic acid, 1-[(*S*)-5-[(aminoiminomethyl)amino]-1-oxo-2-{[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino}pentyl]-4-methyl-, (2*R*,4*R*)-monohydrate;

(2*R*,4*R*)-4-Methyl-1-{N^2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}pipecolic acid, monohydrate CAS RN®: 141396-28-3; UNII: IY90U61Z3S.

DEFINITION

Argatroban contains NLT 98.0% and NMT 102.0% of argatroban ($C_{23}H_{36}N_6O_5S$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

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

• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]

Solution A: 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate

Solution B: Acetonitrile and methanol (500:300)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
20	60	40
35	50	50
50	20	80
60	20	80
60.1	60	40
72.1	60	40

Standard solution: 4 mg/mL of USP Argatroban RS in methanol

Sample solution: 4 mg/mL of Argatroban in methanol

Chromatographic system

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

Mode: LC

Detector: UV 259 nm

Column: 4.6-mm × 25-cm; 3-µm packing L1

Temperatures

Column: 50°

Autosampler: 4°

Flow rate: 0.6 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for both peaks

Relative standard deviation: NMT 1.0% for the sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of argatroban ($C_{23}H_{36}N_6O_5S$) in the portion of Argatroban taken:

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

r_U = sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban from the *Sample solution*

r_S = sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban from the *Standard solution*

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

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

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

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

• **Organic Impurities**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]

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

Sensitivity solution: 2 µg/mL of USP Argatroban RS in methanol

Standard solution: 4 µg/mL each of USP Argatroban RS, USP Argatroban Related Compound A RS, and USP Argatroban Related Compound B RS in methanol

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between (*R*)-argatroban and (*S*)-argatroban, *Standard solution*

Signal-to-noise ratio: NLT 10 for (*R*)-argatroban, *Sensitivity solution*

Relative standard deviation: NMT 5% for all peaks. For argatroban, use the sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban, *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of argatroban related compound A and argatroban related compound B in the portion of Argatroban taken:

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

r_U = peak response of argatroban related compound A or argatroban related compound B from the *Sample solution*

r_S = peak response of argatroban related compound A or argatroban related compound B from the *Standard solution*

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

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

Calculate the percentage of any unspecified impurity in the portion of Argatroban taken:

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

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = sum of the peak responses of (*R*)-argatroban and (*S*)-argatroban from the *Standard solution*

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

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

Acceptance criteria: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound A[a]	0.23	0.15
Argatroban related compound B[b]	0.39	0.15
(*R*)-Argatroban[c]	1.00	—
(*S*)-Argatroban[d]	1.03	—
Any unspecified impurity	—	0.10
Total impurities[e]	—	0.5

[a] (2*R*,4*R*)-1-[N^8-Nitro-N^2-(3-methylquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.

[b] Ethyl (4*R*)-1-[N^8-nitro-L-arginyl]-4-methylpiperidine-2-carboxylate.

[c] (2*R*,4*R*)-4-Methyl-1-{N^2-[((*R*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}pipecolic acid.

[d] (2*R*,4*R*)-4-Methyl-1-{N^2-[((*S*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}pipecolic acid.

[e] Total impurities include specified and unspecified impurities and argatroban related compound C from the test for *Content of Argatroban Related Compound C*.

• **Content of Argatroban Related Compound C**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]

Buffer: 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate

Solution A: Acetonitrile, dehydrated alcohol, and *Buffer* (80:240:680)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Acetonitrile (%)
0	100	0
70	100	0
71	30	70
91	30	70
92	100	0
102	100	0

Sensitivity solution: 4 µg/mL of USP Argatroban Related Compound C RS in methanol

System suitability solution: 10 mg/mL of USP Argatroban RS and 0.1 mg/mL of USP Argatroban Related Compound C RS in methanol

Sample solution: 10 mg/mL of Argatroban in methanol

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

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

Suitability requirements

Resolution: NLT 1.4 between argatroban related compound C and (*R*)-argatroban, *System suitability solution*

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

Relative standard deviation: NMT 2.5% for argatroban related compound C, *System suitability solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of argatroban related compound C in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of argatroban related compound C from the *Sample solution*

r_T = total of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound C[a]	0.94	0.15
(*R*)-Argatroban	1.00	—
(*S*)-Argatroban	1.07	—

[a] (2*R*,4*R*)-1-[N^8-Amino-N^2-(3-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.

• **Content of Stereoisomers**

[Note—It is recommended to keep all solutions containing argatroban at about 4°.]

Mobile phase: Methanol and water (520:480)

Standard solution: 0.16 mg/mL of USP Argatroban RS in methanol

Sample solution: 0.16 mg/mL of Argatroban in methanol

Chromatographic system

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

Mode: LC

Detector: UV 259 nm

Column: 4.6-mm × 25-cm; 3-µm packing L1

Column temperature: 50°

Flow rate: 0.6 mL/min

Injection volume: 10 µL

Run time: NLT 1.4 times the retention time of (*R*)-argatroban

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between (*R*)-argatroban and (*S*)-argatroban

Relative standard deviation: NMT 2.0% for (*R*)-argatroban and (*S*)-argatroban

Analysis

Sample: *Sample solution*

Calculate the percentage of (*R*)-argatroban and (*S*)-argatroban in the portion of Argatroban taken:

$$\text{Result} = [(r_U \text{ or } r_S)/(r_U + r_S)] \times 100$$

r_U = peak response of (*R*)-argatroban from the *Sample solution*

r_S = peak response of (*S*)-argatroban from the *Sample solution*

Acceptance criteria: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, (%)
(*R*)-Argatroban[a]	1.00	63–67
(*S*)-Argatroban[b]	1.06	33–37

[a] (2*R*,4*R*)-4-Methyl-1-{N^2-[(*R*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}pipecolic acid.

[b] (2*R*,4*R*)-4-Methyl-1-{N^2-[(*S*)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}pipecolic acid.

SPECIFIC TESTS

- **Water Determination ⟨921⟩, *Method Ia*:** 3.0%–6.0%
- **Bacterial Endotoxins Test ⟨85⟩:** NMT 2.0 USP Endotoxin Units/mg of argatroban
- **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards ⟨11⟩**

USP Argatroban RS

USP Argatroban Related Compound A RS

(2*R*,4*R*)-1-[N^8-Nitro-N^2-(3-methylquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.
$C_{23}H_{31}N_7O_7S$ 549.60

USP Argatroban Related Compound B RS

Ethyl (4*R*)-1-[N^8-nitro-L-arginyl]-4-methylpiperidine-2-carboxylate dihydrochloride.
$C_{15}H_{28}N_6O_5 \cdot 2HCl$ 445.34

USP Argatroban Related Compound C RS

(2*R*,4*R*)-1-[N^8-Amino-N^2-(3-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.
$C_{23}H_{37}N_7O_5S$ 523.65

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

Topic/Question	Contact	Expert Committee
ARGATROBAN	Documentary Standards Support	SM22020 Small Molecules 2
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 41(3)

Current DocID: GUID-7E80D9CB-CA84-4C40-ACD4-C971E28C02A2_6_en-US

DOI: https://doi.org/10.31003/USPNF_M5830_06_01

DOI ref: sws9f