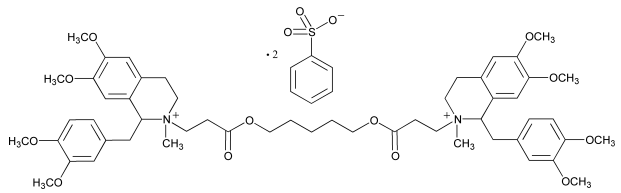
Atracurium Besylate



$C_{65}H_{82}N_2O_{18}S_2$ 1243.48

Isoquinolinium, 2,2′-[1,5-pentanediylbis[oxy(3-oxo-3,1-propanediyl)]]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-, dibenzenesulfonate.

2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium benzenesulfonate, pentamethylene ester [*64228-81-5*].

» Atracurium Besylate contains not less than 96.0 percent and not more than 102.0 percent of $C_{65}H_{82}N_2O_{18}S_2$, calculated on the anhydrous basis. It contains not less than 5.0 percent and not more than 6.5 percent of the *trans-trans* isomer, not less than 34.5 percent and not more than 38.5 percent of the *cis-trans* isomer, and not less than 55.0 percent and not more than 60.0 percent of the *cis-cis* isomer.

Packaging and storage—Preserve in tight, light-resistant containers, in a cold place. [NOTE—Atracurium Besylate is unstable at room temperature.]

USP Reference standards ⟨11⟩**—***USP Atracurium Besylate RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention times of the three main isomeric peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Water, *Method I* ⟨921⟩: not more than 5.0%.

Residue on ignition ⟨281⟩: not more than 0.2%.

Heavy metals, *Method II* ⟨231⟩: 20 µg per g.

Limit of methyl benzenesulfonate—

Buffer solution, Solution A, Solution B, and *Mobile phase*—Prepare as directed in the *Assay*.

Standard solution—Prepare a solution of methyl benzenesulfonate in acetonitrile having a known concentration of about 0.2 mg per mL. Quantitatively dilute a portion of this solution with *Solution A* to obtain a solution having a known concentration of about 1 µg per mL.

Test solution—Transfer about 100 mg of Atracurium Besylate, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Solution A* to volume, and mix.

Resolution solution—Transfer 1 mL of the *Test solution* and 5 mL of a solution containing 0.2 mg of methyl benzenesulfonate per mL to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 217-nm detector and a 4.6-mm × 25-cm column that contains base-deactivated packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	80	20	equilibration
0–5	80	20	isocratic
5–15	80→75	20→25	linear gradient
15–25	75	25	isocratic
25–30	75→55	25→45	linear gradient
30–38	55→0	45→100	linear gradient
38–45	0	100	isocratic

Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the *trans-trans* isomer and methyl benzenesulfonate is not less than 12.0. Chromatograph duplicate injections of the *Standard solution,* and record the peak responses as directed for *Procedure:* the responses for duplicate injections do not differ from each other by more than 12%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the methyl benzenesulfonate peaks: the peak response obtained from the *Test solution* is not greater than that obtained from the *Standard solution.* Not more than 0.01% of methyl benzenesulfonate is found.

Limit of toluene—

Standard solution—Prepare a solution in organic-free water (see *Organic Volatile Impurities* ⟨467⟩, after July 1, 2008, see *Residual Solvents* ⟨467⟩) containing 100 µg of toluene per mL.

Test solution—Dissolve in organic-free water (see *Organic Volatile Impurities* ⟨467⟩, after July 1, 2008, see *Residual Solvents* ⟨467⟩) an accurately weighed portion of the material to be tested to obtain a final solution having a known concentration of about 20 mg of the test material per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 30-m fused silica analytical column coated with a 5-µm chemically cross-linked G27 stationary phase and a 0.53-mm × 5-m silica guard column deactivated with phenylmethyl siloxane. The carrier gas is helium with a linear velocity of about 35 cm per second. [NOTE—When a makeup gas is used, nitrogen is recommended.] The injection port temperature and the detector temperature are maintained at 70° and 260°, respectively. The column temperature is programmed as follows. Initially, the column temperature is maintained at 35° for 5 minutes, then increased at a rate of 8° per minute to 175°, followed by an increase at a rate of 35° per minute to 260°, and maintained at 260° for at least 16 minutes. Inject the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation of the toluene peak from replicate injections is not more than 15%.

Procedure—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks: the toluene peak from the *Test solution* is not greater than the toluene peak obtained from the *Standard solution*. Not more than 0.5% of toluene is found.

Chromatographic purity—

Buffer solution, Solution A, Solution B, and *Mobile phase*—Proceed as directed in the *Assay.*

Standard solution—Transfer 1.0 mL of the *Standard preparation,* prepared as directed in the *Assay,* to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix.

Test solution—Use the *Assay preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay.* Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the responses of the *cis-cis* isomers from not fewer than two injections do not differ by more than 10%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses, except the three main isomeric peaks. Calculate the percentage of each impurity in the portion of Atracurium Besylate taken by the formula:

$$10{,}000(1/F)(C/W)(r_i / r_S)$$

in which F is the relative response factor of the impurity peak, which is 1.9 for laudanosine and 1.0 for all other unidentified impurities; C is the concentration, in mg per mL, of the *cis-cis* isomer in the *Standard solution; W* is the weight, in mg, of Atracurium Besy-

late taken to prepare the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for the *cis-cis* isomer obtained from the *Standard solution:* not more than 0.5% of laudanosine is found, not more than 1.0% of any other individual impurity is found, and not more than 3.5% of total impurities is found. [NOTE—For identification purposes, the relative retention time for laudanosine is about 0.3.]

Organic volatile impurities, *Method I* ⟨467⟩**—**meets the requirements.

(Official until July 1, 2008)

Assay—

Buffer solution—Transfer about 10.2 g of monobasic potassium phosphate to a 1000-mL volumetric flask, and dissolve in about 950 mL of water. While stirring, adjust with phosphoric acid to a pH of 3.1, dilute with water to volume, and mix.

Solution A—Prepare a mixture of *Buffer solution,* acetonitrile, and methanol (75 : 20 : 5).

Solution B—Prepare a mixture of *Buffer solution,* methanol, and acetonitrile (50 : 30 : 20).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Atracurium Besylate RS in *Solution A* to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Transfer about 100 mg of Atracurium Besylate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Solution A* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains base-deactivated packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	80	20	equilibration
0–5	80	20	isocratic
5–15	80→40	20→60	linear gradient
15–25	40	60	isocratic
25–30	40→0	60→100	linear gradient

Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the *trans-trans* isomer and the *cis-trans* isomer and between the *cis-trans* isomer and the *cis-cis* isomer is not less than 1.1; and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—For identification purposes, the relative retention times are about 0.8, 0.9, and 1.0 for the *trans-trans* isomer, the *cis-trans* isomer, and the *cis-cis* isomer, respectively.]

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the three isomeric peaks. Calculate the quantity, in mg, of $C_{65}H_{82}N_2O_{18}S_2$ in the portion of Atracurium Besylate taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Atracurium Besylate RS in the *Standard preparation;* and r_U and r_S are the sums of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer obtained from the *Assay preparation* and the *Standard preparation,* respectively.