Identification—

A: *Ultraviolet Absorption* ⟨197U⟩—

Medium: a mixture of methanol and water (1:1).

Solution—Transfer 5.0 mL of the *Assay preparation* and 5.0 mL of the *Standard preparation,* prepared in the *Assay,* to separate 50-mL volumetric flasks, dilute with *Medium* to volume, and mix.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Uniformity of dosage units ⟨905⟩**—**

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩**—**

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH ⟨791⟩**:** between 3.5 and 7.0.

Sedimentation—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS— Transfer 50 mL of well-mixed Oral Suspension to a glass-stoppered graduated cylinder, and allow to stand for 16 hours. Measure the volume, if any, of clear liquid obser ved in the cylinder: not more than 1 mL of clear liquid is found.

Related compounds—Using the chromatograms of the *Resolution solution,* the *Standard preparation,* and the *Assay preparation* obtained in the *Assay,* calculate the percentage of atovaquone-related compounds, based on the labeled strength of atovaquone, by the formula:

$$\frac{(25,000/3)C(r_i/r_S)100D}{SF_iL}$$

in which *C* is the concentration, in mg per mL, of USP Atovaquone RS in the *Standard preparation; D* is the density of Oral Suspension, in g per mL (1.04 g per mL at 20 ° to 25 °); *S* is the weight, in g, of Oral Suspension taken to prepare the *Assay preparation; L* is the labeled amount, in mg per mL, of atovaquone in the Oral Suspension; F_i is the response factor of an individual atovaquone related compound relative to the response of atovaquone, specifically, 1.08 for any peak obser ved at a relative retention time of about 0.65, 0.85 for any peak observed at a retention time corresponding to that of atovaquone related compound A, as determined from the chromatogram of the *Resolution solution,* and 1.0 for any other related compound peak; r_i is the individual peak response of an atovaquone related compound, if any, in the chromatogram of the *Assay preparation;* and r_S is the peak response of atovaquone in the chromatogram of the *Standard preparation.* Disregard any peak having a relative retention time of about 0.3, which is due to photodegradation during preparation of the *Assay preparation.* Not more than 0.5% of an atovaquone related compound with a relative retention time of about 0.65 is found; not more than 1.0% of atovaquone related compound A is found; not more than 0.3% of an atovaquone related compound with a relative retention time of about 0.88 is found; not more than 0.2% of any other atovaquone related compound is found; and the sum of all related compounds is not more than 2.0%.

Assay—

Mobile phase—Prepare a mixture of acetonitrile, water, methanol, and phosphoric acid (480:360:160:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Prepare a solution in 0.1 M methanolic sodium hydroxide containing about 0.09 mg of USP Atovaquone RS and 0.01 mg of USP Atovaquone Related Compound A RS per mL. Store in a low-actinic glass container.

Standard preparation—Transfer about 30 mg of USP Atovaquone RS, accurately weighed, to a low-actinic 10-mL volumetric flask, and add 2 mL of water and 6 mL of 0.1 M methanolic sodium hydroxide. Sonicate for about 5 minutes or until the material has dissolved. Allow to cool, dilute with 0.1 M methanolic sodium hydroxide to volume, and mix. T ransfer 3.0 mL of this solution to a low-actinic 100-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. [NOTE—Minimize exposure of this solution to light.]

Assay preparation—Transfer approximately 5.2 g of the well-mixed Oral Suspension, accurately weighed, to a low-actinic 250-mL volumetric flask. Add 50 mL of water, swirl for about 5 minutes, add 150 mL of 0.1 M methanolic sodium hydroxide, and sonicate for about 15 minutes. Allow to cool, dilute with 0.1 M methanolic sodium hydroxide to volume, and mix. Immediately filter a 20-mL portion, discarding the first 5 mL of the filtrate. Transfer 3.0 mL of the clear filtrate to a low-actinic 100-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. [NOTE—Minimize exposure of this solution to light.]

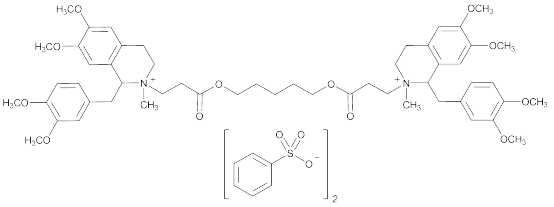
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 12.5-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution,* and record the peak areas as directed for *Procedure:* the relative retention times are about 0.86 for atovaquone related compound A and 1.0 for atovaquone. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation,* the *Resolution solution,* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of atovaquone ($C_{22}H_{19}ClO_3$) in each mL of the Oral Suspension taken by the formula:

$$(25,000/3)(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Atovaquone RS in the *Standard preparation; V* is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation;* and r_U and r_S are the atovaquone peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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 per cent of $C_{65}H_{82}N_2O_{18}S_2$, calculated on the anhydrous basis. It contains not less than 5.0 per cent and not more than 6.5 per cent of the *trans-trans* isomer, not less than 34.5 per cent 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 acetonitrile 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 *Residual Solvents* ⟨467⟩) containing 100 µg of toluene per mL.

Test solution—Dissolve in organic-free water (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 Besylate 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.]

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.

Atracurium Besylate Injection

» Atracurium Besylate Injection is a sterile solution containing not less than 90.0 per cent and not more than 115.0 per cent of the labeled amount of atracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$). It contains an amount of the *trans-trans*-isomer equivalent to not less than 5.0 per cent and not more than 6.5 per cent of the labeled amount of atracurium besylate, an amount of the *cis-trans*-isomer equivalent to not less than 34.5 per cent and not more than 38.5 per cent of the labeled amount of atracurium besylate, and an amount of the *cis-cis*-isomer equivalent to not less than 55.0 percent and not more than 60.0 per cent of the labeled amount of atracurium besylate.

NOTE—The Injection is unstable at room temperature. Store all samples in the refrigerator. Analyze all preparations as soon as possible, or use a refrigerated injector.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of T ype I glass, in a refrigerator, and protect from freezing. Protect from light.

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

Identification—The retention times of the peaks of the three atracurium besylate isomers in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 5.56 USP Endotoxin Units per mg of atracurium besylate.

Sterility ⟨71⟩**—**It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩**:** between 3.00 and 3.65.

Related compounds—

Buffer solution, Solution A, Solution B, Mobile phase, and *Standard preparation*—Proceed as directed in the *Assay* under *Atracurium Besylate.*

System suitability solution—Heat a portion of the *Standard preparation* at 90° for 30 minutes, and immediately chill to about 5°.

Diluted standard solution—Dilute a portion of the *Standard preparation* quantitatively, and stepwise if necessar y, with *Solution A* to obtain a solution having a known concentration of about 0.02 mg per mL.

Test solution—Use the *Assay preparation.*

Chromatographic system—Prepare as directed for *Chromatographic system* in the *Assay.* Chromatograph the *System suitability solution* and the *Diluted standard solution,* record the chromatograms, and measure the responses for the degradation products by comparing the peak responses of the *System suitability solution* to those of the *Diluted standard solution* as directed for *Procedure:* the retention times relative to the atracurium besylate *cis-cis*-isomer are about 0.22 for the acidic compound; 0.29 for laudanosine; 0.44 and 0.50 for the *trans-* and *cis*-isomers, respectively, of the hydroxy compound; and about 1.28 and 1.33 for the *trans-* and *cis*-isomers, respectively, of the monoacr ylate.

Procedure—Separately inject equal volumes (about 20 µL) of the *Diluted standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses, except the peak due to benzenesulfonic acid occurring at a retention time of about 0.08 relative to the atracurium besylate *cis-cis*-isomer. Calculate the per centage of each impurity in the portion of *Test solution* taken by the formula:

$$100(C/M)(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Atracurium Besylate RS in the *Diluted standard solution; M* is the concentration of atracurium besylate, in mg per mL, in the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_s is the sum of the responses of all the peaks obtained from the *Diluted standard solution:* not more than 6.0% of the acidic compound, not more than 6.0% of the combined *cis-* and *trans*-isomers of the hydroxy compound, not more than 3.0% of laudanosine, not more than 3.0% of the combined *cis-* and *trans*-isomers of the monoacr ylate, and not more than 0.1% of any other impurity is found; and not more than 15.0% of total impurities is found.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Buffer solution, Solution A, Solution B, Mobile phase, and *Standard preparation*—Proceed as directed in the *Assay* under *Atracurium Besylate.*

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of atracurium besylate, to a 50-mL volumetric flask, dilute with *Solution A* to volume, and mix.