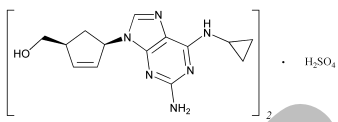
BRIEFING

Abacavir Sulfate. A new USP International Standards monograph, based on the submitted data, is being proposed. The HPLC procedures used in the test for *Related compounds* are based on analyses perfomed with the Hypersil BDS C8 brand of L7 column. The HPLC procedures used in the *Assay* are based on analyses performed with the Symmetry C8 brand of column containing 5-µm packing L7. The HPLC procedures used in the test for *Enantiomeric purity* are based on analyses performed with the Chiralpak-AD brand of L51 column.

(MD-AA: L. Santos) RTS—C46971

Add the following:

■Abacavir Sulfate



$(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$ 670.74

(1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol sulfate (2 : 1) [*188062-50-2*].

» Abacavir Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at controlled room temperature.

USP Reference standards ⟨11⟩—*USP Abacavir Sulfate RS. USP Abacavir Sulfate Racemic RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩—

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.*

C: *Sulfates* ⟨191⟩—It meets the requirements of the test for *Sulfate*.

Water, *Method I* ⟨921⟩**:** not more than 1.0%.

Residue on ignition ⟨281⟩**:** not more than 0.3%, a 1.0-g test specimen being used.

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Limit of the abacavir enantiomer—

Mobile phase—Prepare a degassed mixture of *n*-hexane, dehydrated alcohol, and methanol (800 : 100 : 100). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock solution—Transfer about 20 mg of USP Abacavir Sulfate RS, accurately weighed, into a 50-mL volumetric flask. Add 5 mL of methanol, and dilute with *Mobile phase* to volume to obtain a solution having a known concentration of about 0.4 mg per mL.

Standard solution—Dilute an accurately measured volume of *Standard stock solution,* stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

System suitability solution—Transfer about 5 mg of USP Abacavir Sulfate Racemic RS to a 10-mL volumetric flask, and add 1 mL of methanol. Dissolve in and dilute with *Mobile phase* to volume.

Test solution—Transfer about 50 mg of Abacavir Sulfate, accurately weighed, to a 100-mL volumetric flask. Dissolve in 10 mL of methanol, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L51. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* [NOTE—The relative retention times are about 0.8 for the abacavir enantiomer and 1.0 for abacavir.] the resolution, *R,* between the abacavir enantiomer and abacavir is not less than 2.5. Chromatograph the

Standard solution, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of the abacavir enantiomer in the portion of Abacavir Sulfate taken by the formula:

$$100 C_S / C_U (r_U / r_S)$$

in which C_S and C_U are the concentrations, in µg per mL, of the *Standard solution* and the *Test solution,* respectively; and r_U and r_S are the peak responses of the abacavir enantiomer and abacavir sulfate obtained from the *Test solution* and the *Standard solution,* respectively: not more than 0.2% of the abacavir enantiomer is found.

Related compounds—

Solution A—Dissolve 4.0 g of sodium dihydrogen orthophosphate dihydrate in 1000 mL of water, and adjust with orthophosphoric acid to a pH of 3.0.

Solution B—Use acetonitrile.

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 solution—Dissolve an accurately weighed quantity of USP Abacavir Sulfate RS in water. Dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.001 mg per mL.

System suitability solution—Transfer a weighed quantity of USP Abacavir Sulfate RS to a volumetric flask, dissolve in and dilute with water to volume, and mix to obtain a solution having a concentration of 0.1 mg per mL.

Test solution—Transfer about 50 mg of Abacavir Sulfate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Table 1

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–20	98→88	2→12	linear gradient
20–30	88→40	12→60	linear gradient
30–35	40	60	isocratic
35–36	40→98	60→2	linear gradient

Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure*: the column efficiency is not less than 25,000 theoretical plates; and the tailing factor is not more than 2.0. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatogram. Identify the

impurities using the relative retention times specified in *Table 2* below, and measure the peak responses.

Table 2

Name	Approximate Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
Descyclopropyl-abacavir[1]	0.64	1.6	0.1
Abacavir	1.0	—	—
Dihydro abacavir[2]	1.12	0.87	0.1
Abacavir penultimate[3]	1.16	1.5	0.1
O-Pyrimidinyl abacavir[4]	1.43	0.93	0.15
Unknown impurities	—	1.0	0.1
Total impurity	—	—	0.5

[1] (1*S*,4*R*)-4-[2,6-Diamino-9*H*-purin-9-yl]-2-cyclopentene-1-methanol hydrochloride
[2] (1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopentane-1-methanol sulfate (1 : 1)
[3] (1*S*,4*R*)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-cyclopentene-1-methanol hydrochloride
[4] (1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-1-(2,5-diamino-6-chloro-4-pyrimidinyloxy)methyl-2-cyclopentene

Calculate the percentage of each impurity in the portion of Abacavir Sulfate taken by the formula:

$$(100/F)(C_S/C_U)(r_U/r_S)$$

in which F is the relative response factor for each impurity, as listed in *Table 2;* C_S and C_U are the concentrations, in mg per mL, of USP Abacavir Sulfate RS in the *Standard solution* and the *Test solution,* respectively; r_U is the peak area for each impurity obtained from the *Test solution;* and r_S is the peak area of abacavir obtained from the *Standard solution*.

Content of sulfate—

Test solution—Dissolve about 300 mg of Abacavir Sulfate, accurately weighed, in about 50 mL of water.

Procedure—Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically, using a reference-combined appropriate glass electrode. Each mL of 0.1 N sodium hydroxide VS is equivalent to a 4.9 mg of sulfate: between 13.6% and 15.0% of sulfate, calculated on the as-is basis, is found.

Assay—

Buffer—Dissolve 4.0 g of sodium dihydrogen orthophosphate dihydrate in 1000 mL of water, and adjust with orthophosphoric acid to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer* and acetonitrile (90 : 10). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩)

Standard preparation—Dissolve an accurately weighed quantity of USP Abacavir Sulfate RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay preparation—Transfer about 50 mg of Abacavir Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Further dilute with water to obtain a solution having a concentration of about 0.05 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the abacavir peak is not less than 2000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.0%.

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 abacavir peak. Calculate the percentage of $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$ in the portion of Abacavir Sulfate taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of Abacavir Sulfate in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Description and solubility—

Abacavir Sulfate: White to off-white, crystalline powder. Soluble in water; slightly soluble in methanol. ■