

BRIEFING

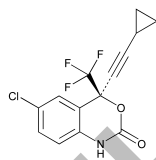
Efavirenz. A new USP Salmous Standards monograph, based on the submitted data is being proposed. The HPLC procedure used in the test for *Related compounds* and in the *Assay* is based on analysis performed with a Hypersil BDS brand of L1 column. The HPLC procedure used in the test for *Limit of efavirenz enantiomer* is based on the analysis performed with a Chiralpak-AD brand of L51 column. Typical retention times for efavirenz based on the assay method is 10.9 minutes and for related compound method is 16.7 minutes.

(IH: L. Santos) RTS—C44999

Add the following:

■ **Efavirenz**

v. 1 Authorized November 1, 2007



C₁₄H₉ClF₃NO₂ 315.67

2*H*-3,1-Benzoxazin-2-one, 6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-, (*S*)-
(*S*)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one [154598-52-4].

» Efavirenz contains not less than 98.0 percent and not more than 102.0 percent of C₁₄H₉ClF₃NO₂, calculated on the anhydrous basis.

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

USP Reference standards <11>—*USP Efavirenz RS*. *USP Efavirenz 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*.

Water, Method I <921>: not more than 0.5%.

Residue on ignition <281>: not more than 0.2%, a 1.0-g test specimen being used.

Heavy metals, Method II <231>: 0.002%.

Limit of efavirenz enantiomer—

Mobile phase—Prepare a mixture of *n*-hexane, dehydrated alcohol, and diethylamine (980 : 20 : 2). Mix, degas, and make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

Standard solution—Dissolve an accurately weighed quantity of USP Efavirenz RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

System suitability solution—Dissolve an accurately weighed quantity of USP Efavirenz Racemic RS in *Mobile phase*, and dilute with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

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

Chromatographic system (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 252-nm detector and a 4.6-mm × 25-cm column that contains packing L51. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 25°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for (*S*)-enantiomer (efavirenz) and 1.53 for (*R*)-enantiomer; the resolution, *R*, between (*S*)-enantiomer (efavirenz) and (*R*)-enantiomer is not less than 5.0. 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%.

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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 (*R*)-enantiomer in the portion of Efavirenz taken by the formula:

$$100(C_s/C_v)(r_v/r_s)$$

in which C_s and C_v are the concentrations, in mg per mL, of efavirenz in the *Standard solution* and the *Test solution*, respectively; r_v and r_s are the peak responses of (*R*)-enantiomer and (*S*)-enantiomer (efavirenz) obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.2% of (*R*)-enantiomer is found.

Related compounds—

Solution A—Dissolve 800 mg of ammonium acetate in 1000 mL of water, and adjust with 0.5% ammonia solution to a pH of 7.5 ± 0.05 .

Solution B—Use acetonitrile.

Diluent—Mix *Solution A* and *Solution B* in the ratio of 50 : 50.

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 Efavirenz RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.001 mg per mL.

System suitability solution—Dissolve an accurately weighed quantity of USP Efavirenz RS in *Diluent*, and dilute with *Diluent*, to obtain a solution having a known concentration of about 0.5 mg per mL.

Test solution—Transfer an accurately weighed quantity of about 100 mg of Efavirenz to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 252-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	<i>Solution A</i> (%)	<i>Solution B</i> (%)	Elution
0–25	50	50	isocratic
25–40	50→20	50→80	linear gradient
40–55	20	80	isocratic
55–60	20→50	80→50	linear gradient

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 8000 theoretical plates; and the tailing factor is not more than 1.8. 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 chromatograms. Identify the impurities based on relative retention times given in the accompanying table, and measure the peak responses. Calculate the percentage of each impurity in the portion of Efavirenz taken by the formula:

$$(100/F)(C_s/C_v)(r_v/r_s)$$

in which F is the response factor for each impurity against Efavirenz as listed in the table below; C_s is the concentration, in mg per mL, of USP Efavirenz RS in the *Standard solution*; C_v is the concentration, in mg per mL, of Efavirenz in the *Test solution*; r_v is the peak area for each impurity obtained from the *Test solution*; and r_s is the peak area of efavirenz obtained from the *Standard solution*.

Compound	Approximate Relative Retention Time	Response Factor (<i>F</i>)	Limit (%)
Efavirenz impurity A ¹	0.80	0.83	0.1
Efavirenz	1.0	—	—
Efavirenz impurity B ²	1.90	0.73	0.1
Unknown impurities	—	1.0	0.1
Total impurity	—	—	0.5

¹ (S)-5-Chloro- α -(cyclopropylethynyl)-2-amino- α -(trifluoromethyl)benzene methanol.

² (S)-5-Chloro- α -(cyclopropylethynyl)-2-[4'-methoxybenzoylamino]- α -(trifluoromethyl)benzene methanol.

Assay—

Buffer—Dissolve 800 mg of ammonium acetate in 1000 mL of water, and adjust with dilute ammonia solution to a pH of 7.5.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer* and acetonitrile (50 : 50). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Efavirenz RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.10 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of about 50 mg of Efavirenz to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Further dilute an aliquot of this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 252-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the efavirenz peak is not less than 5000 theoretical plates; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injection is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the efavirenz peak. Calculate the percentage of C₁₄H₉ClF₃NO₂ in the portion of Efavirenz taken by the formula:

$$100(C_s/C_v)(r_u/r_s)$$

in which C_s and C_v are the concentration, in mg per mL, of efavirenz 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—

Efavirenz: White to off-white, crystalline powder. Soluble in methanol.■