

BRIEFING

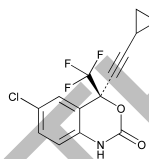
Efavirenz. This monograph was posted on the USP website as a draft USP Pending Standard for public comment. No comments were received. The MD-AA Expert Committee reviewed the draft and approved the monograph as an Authorized USP Pending Standard. The HPLC procedure used in the test for *Related compounds* and in the *Assay* is based on analysis performed with the Hypersil BDS brand of L1 column. The HPLC procedure used in the test for *Limit of efavirenz enantiomer* is based on analysis performed with the Chiralpak-AD brand of L51 column. The typical retention time for efavirenz, based on the *Assay* method, is 10.9 minutes; the typical retention time for efavirenz, based on the *Related compounds* method, is 16.7 minutes.

(MD-AA: B. Davani) RTS—C58871

Add the following:

■ **Efavirenz**

v.1 Authorized September 20, 2007



C₁₄H₉ClF₃NO₂ 315.67

(*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 for (*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 shown in *Table 1*.

Table 1

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 the relative retention times given in *Table 2*, 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 *Table 2*; 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_U 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*.

Table 2

Compound	Approximate	Response	
	Retention Time	Factor (F)	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 \times 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 injections 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:

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

in which C_S and C_U 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. ■