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Enalaprilat Injection

» Enalaprilat Injection is a sterile solution of enalaprilat in a suitable vehicle for injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of enalaprilat ($C_{18}H_{24}N_2O_5$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩—

USP Benzyl Alcohol RS
USP Enalaprilat RS
USP Enalapril Maleate RS

Identification—

A: The retention time of the enalaprilat peak in the chromatogram of the *Assay preparation* corresponds to that of the corresponding peak in the chromatogram of the *Standard preparation,* obtained as directed in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩—It contains no more than 280 USP Endotoxin Units per mg of enalaprilat.

Sterility Tests ⟨71⟩**:** meets the requirements.

pH ⟨791⟩**:** between 6.5 and 7.5.

Particulate Matter in Injections ⟨788⟩**:** meets the requirements for small-volume injections.

Change to read:

▲**Osmolality and Osmolarity** ⟨785⟩

Osmolality:▲ *(Official 1-Aug-2022)* between 276 to 305 mOsm per kg.

Related compounds—

Diluent—Prepare a mixture of water and acetonitrile (84:16).

Buffer solution—Dilute 3.4 mL of phosphoric acid with water to 1 L. Adjust with potassium hydroxide to a pH of 2.5.

Solution A—Use a mixture of *Buffer solution* and acetonitrile (84:16).

Solution B—Use acetonitrile.

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

Stock solution A—Dissolve 20 mg of USP Enalaprilat RS in a 100-mL volumetric flask with approximately 80 mL of *Diluent*. Heat at 80° for 24 hours to generate enalaprilat related compound A. Cool the solution to room temperature, and dilute with *Diluent* to volume.

Stock solution B—Dissolve 10 mg of USP Enalapril Maleate RS in a 200-mL volumetric flask, and dilute with *Diluent* to volume.

Stock solution C—Dissolve accurately weighed quantities of benzyl alcohol, benzaldehyde, and benzoic acid in *Diluent*. Dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of 0.1 mg per mL of each of the three substances.

System suitability solution—Pipet 4.0 mL of *Stock solution A,* 5 mL of *Stock solution B,* and 5 mL of *Stock solution C* into a 25-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution containing about 0.0032 mg per mL of enalaprilat related impurity A, 0.01 mg per mL of enalapril, and 0.02 mg per mL of each of benzyl alcohol, benzaldehyde, and benzoic acid, respectively.

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 12.5 mg of enalaprilat, to a 25-mL volumetric flask. Dilute with *Diluent* to volume, and mix.

Diluted test solution—Dilute the *Test solution* (1 in 100) with *Diluent* to obtain a solution having a known concentration of 0.005 mg per mL (corresponds to 1% of the *Test solution*).

Chromatographic system (see *Chromatography* ⟨621⟩)—The chromatograph is equipped with 215-nm detector and a 4.6-mm × 15-cm, 5-µm column that contains packing L1. The column temperature is maintained at 60°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows:

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–5	97.0	3.0	isocratic
5–20	97→77.5	3→22.5	linear gradient
20–25	77.5→10	22.5→90	linear gradient

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
25–25.01	10→97	90→3.0	step gradient
25.01–30	97	3.0	re-equilibrium

Chromatograph the *System suitability solution* and the *Diluted test solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between enalapril maleate and enalaprilat related impurity A is not less than 1.2; the capacity factor for enalaprilat is not less than 1.5; and the relative standard deviation of the enalaprilat peak in replicate injections of the *Diluted test solution* is not more than 2.0%. *Procedure*—Separately inject equal volumes (about 20 µL) of the *Diluted test solution* and the *Test solution* into the chromatograph, record the chromatogram, and measure the peak response for enalaprilat in the *Diluted test solution* and all of the peak responses from the *Test solution* that do not correspond to enalaprilat, benzyl alcohol, benzoic acid, benzaldehyde, and benzyl alcohol related compounds (see *Table 1* for RRT). Calculate the percentage of specified and unspecified impurities using the formula:

$$(r_i/r_S)$$

in which r_i is the peak response for each specified impurity in the *Test solution;* and r_S is the peak response for enalaprilat in the *Diluted test solution:* the impurities meet the specified limits in *Table 1*.

Table 1

Component	Relative Retention Time (minutes)	Limit (w/w, %)
Enalaprilat heat degradation product	0.6 vs enalaprilat	0.5
Enalaprilat	1 vs enalaprilat	—
Benzyl alcohol	1 vs benzyl alcohol	—
Benzyl alcohol related unknown impurity 1	1.2 vs benzyl alcohol	—
Benzoic acid	1.4 vs benzyl alcohol	—
Benzyl alcohol related unknown impurity 2	1.7 vs benzyl alcohol	—
Benzaldehyde	2.1 vs benzyl alcohol	—
Enalapril maleate	4.7 vs enalaprilat	0.25
Enalaprilat related impurity A	5.1 vs enalaprilat	1.0
Any other unspecified individual impurity	—	0.10
Total impurities	—	2.0

Benzyl alcohol content *(if present)*—

Buffer solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Benzyl Alcohol RS in *Mobile phase* to obtain a solution having a known concentration of 0.72 mg per mL.

Test solution—Use the *Assay preparation,* prepared as directed in the *Assay*.

Procedure—Proceed as directed in the *Assay*. Calculate the percentage of benzyl alcohol, based on the label claim, in the volume of Injection taken by the formula:

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

in which C_S is the concentration, in mg per mL, of USP Benzyl Alcohol RS in the *Standard solution;* C_U is the concentration, in mg per mL, of benzyl alcohol in the *Test solution;* and r_U and r_S are the benzyl alcohol peak responses obtained from the *Test solution* and the *Standard solution,* respectively: between 75.0% and 120.0% of the labeled amount is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products ⟨1⟩*.

Assay—

Buffer solution—Prepare a solution of 0.05 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (84 : 16). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

System suitability preparation—Use the *Standard preparation.*

Standard preparation—Dissolve an accurately weighed quantity of USP Enalaprilat RS and USP Benzyl Alcohol RS in *Buffer solution,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL of enalaprilat and 0.72 mg per mL of benzyl alcohol.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of enalaprilat, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 215-nm and 258-nm detector (use 215 nm as the initial wavelength, and switch to 258 nm after the elution of enalaprilat and before the elution of benzyl alcohol) and a 4.6-mm × 15-cm, 5-µm column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 60°. Chromatograph the *System suitability preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between benzyl alcohol and enalaprilat is not less than 3.0; the tailing factor for benzyl alcohol and the enalaprilat peaks 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* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the quantity, in percentage of the label claim, of enalaprilat ($C_{18}H_{24}N_2O_5$) in the portion of Injection taken by the formula:

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

in which C_S is the concentration, in mg per mL, of USP Enalaprilat RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of enalaprilat in the *Assay preparation*, based on the label claim; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ENALAPRILAT INJECTION	Documentary Standards Support	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

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