$C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ in the portion of Enalapril Maleate taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Enalapril Maleate Tablets

» Enalapril Maleate Tablets contain not less than 90.0 percent and not more than 110.0 per cent of the labeled amount of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Enalapril Maleate RS
USP Enalaprilat RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

Dissolution ⟨711⟩**—**

Medium: pH 6.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ dissolved as directed for *Procedure for content uniformity* under *Uniformity of dosage units* except to use pH 6.8 phosphate buffer instead of the *Buffer solution* to prepare the *Standard preparation,* to use a filtered portion of the solution under test as the *Test preparation*, and to make any necessary modifications for appropriate sample and standard concentrations.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Buffer solution and *Mobile phase*—Prepare as directed in the *Assay*.

Standard preparation—Transfer about 10 mg of USP Enalapril Maleate RS to a 100-mL volumetric flask. Add about 50 mL of *Buffer solution*, shake, and use sonication if necessary to dissolve. Dilute with *Buffer solution* to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Enalapril Maleate RS per mL.

Test preparation—Transfer one Tablet to a volumetric flask of capacity such that, when filled to volume, will produce a solution having a concentration of about 0.1 mg of enalapril maleate per mL. Proceed as directed for *Assay preparation* in the *Assay*, beginning with "Add a volume of *Buffer solution* that is about one-half the nominal volume of the flask."

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains 5- µm packing L7. The column temperature is maintained at 50 °, and the flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 300 theoretical plates; the tailing factor is not more than 2.0; the capacity factor, k', is not less than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—The enalapril peak tailing factor may be minimized by controlling the column temperature between 45 ° and 50 ° and by raising the pH of the aqueous component of the *Mobile phase* from 2.2 to 2.6; the capacity factor may be increased by decreasing the amount of acetonitrile in the *Mobile phase*.]

Procedure—Separately inject equal volumes (about 50 µL) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ in the Tablet taken by the formula:

$$(TC/D)(r_U / r_S)$$

in which T is the labeled quantity, in mg, of enalapril maleate in the Tablet; C is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Standard preparation; D* is the concentration, in mg per mL, of enalapril maleate in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and r_U and r_S are the enalapril peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively.

Related compounds—

Buffer solution, *Mobile phase*, *Enalaprilat standard solution*, *Enalapril diketopiperazine solution*, *System suitability solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Test preparation—Use the *Assay preparation*.

Related compounds standard solution—Transfer 1.0 mL of the *Standard preparation* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation,* the *Test preparation*, the *Related compounds standard solution*, and the *Buffer solution*, into the chromatograph, record the chromatograms, and measure the responses for all of the peaks in the *Test preparation* greater than 0.1% of the response of the enalapril peak that are not observed in the *Buffer solution*. Calculate the percentage of anhydrous enalaprilat (as enalapril maleate) present in the portion of Tablets taken by the formula:

$$(492.52/348.39)(CV/N)(r_U / r_S)(100/L)$$

in which 492.52 and 348.39 are the molecular weights of enalapril maleate and anhydrous enalaprilat, respectively; C is the concentration, in mg per mL, of USP Enalaprilat RS in the *Standard preparation; V* is the nominal capacity, in mL, of the volumetric flask containing the *Test preparation; N* is the number of Tablets taken for the *Test preparation;* r_U and r_S are the enalaprilat peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively; and L is the labeled amount of enalapril maleate in the Tablet.

Calculate the percentage of enalapril diketopiperazine (as enalapril maleate) present in the portion of Tablets taken by the formula:

$$(492.52/358.44)(C'V/N)(r_U /1.25\ r_S)(100/L)$$

in which 492.52 and 358.44 are the molecular weights of enalapril maleate and enalapril diketopiperazine, respectively; C' is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Related compounds standard solution; V* is the nominal capacity, in mL, of the volumetric flask containing the *Test preparation; N* is the number of Tablets taken for the *Test preparation;* r_U is the enalapril diketopiperazine peak response obtained from the *Test preparation;* 1.25 is the response for enalapril diketopiperazine relative to that for enalapril maleate; r_S is the enalapril peak response obtained from the *Related compounds standard solution;* and L is the labeled amount, in mg, of enalapril maleate in the Tablet.

Calculate the percentage of any other related compound by the formula:

$$(C'V/N)(r_R / r_S)(100/L)$$

in which r_R is the sum of the responses of any related compound, other than those from maleic acid, enalapril, enalaprilat, and enalapril diketopiperazine obtained from the *Test preparation;* r_S is the enalapril peak response obtained from the *Related compounds standard solution;* and C', V, N, and L are as defined

above: the sum of all related compounds including those from enalaprilat and enalapril diketopiperazine is not greater than 5.0%.

Assay—

Buffer solution—Dissolve 1.38 g of monobasic sodium phosphate in about 800 mL of water. Adjust with phosphoric acid to a pH of 2.2, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Enalaprilat standard solution—Dissolve an accurately weighed quantity of USP Enalaprilat RS in water to obtain a solution having a known concentration of about 0.4 mg per mL.

Enalapril diketopiperazine solution—Carefully place about 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at about one-half the maximum hot plate temperature setting to melt the solid. When melting is obser ved, (after 5 to 10 minutes of heating), immediately remove the beaker from the hot plate, and allow it to cool. [NOTE—Avoid over heating beyond the melting initially obser ved to prevent heat-induced degradation, which would give rise to a brown color.] To the cooled residue in the beaker add 50 mL of acetonitrile and sonicate for a few minutes to dissolve the residue. The solution typically contains, in each mL, between 0.2 mg and 0.4 mg of enalapril diketopiperazine.

Standard preparation—Transfer about 20 mg of USP Enalapril Maleate RS, accurately weighed, to a 100-mL volumetric flask. Transfer 0.5 mL of *Enalaprilat standard solution* to the flask, and add about 50 mL of *Buffer solution* to dissolve, using sonication if necessary. Dilute with *Buffer solution* to volume, and mix to obtain a solution having known concentrations of about 0.2 mg of USP Enalapril Maleate RS per mL and 0.002 mg of USP Enalaprilat RS per mL.

System suitability solution—Transfer 0.5 mL of *Enalapril diketopiperazine solution* to a 25-mL volumetric flask, dilute with *Standard preparation* to volume, and mix.

Assay preparation—Transfer not fewer than 10 T ablets to a volumetric flask of capacity such that, when filled to volume, will produce a solution having a concentration of about 0.2 mg of enalapril maleate per mL. Add a volume of *Buffer solution* that is about one-half the nominal volume of the flask, sonicate for 15 minutes, and shake by mechanical means for 30 minutes. Dilute with *Buffer solution* to volume, shake well, and sonicate for 15 minutes. Pass through a filter of 0.45- µm or finer porosity, discarding the first portion of the filtrate.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains 5- µm packing L7. The column temperature is maintained at 50 °, and the flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.3 for maleic acid, 0.5 for enalaprilat, 1.0 for enalapril, and 1.5 for enalapril diketopiperazine. [NOTE—A peak response for heat-induced degradation product of enalapril diketopiperazine (if present with relative retention time about 1.2) is not greater than 15% of the response for enalapril diketopiperazine.] The column efficiency is not less than 1000 theoretical plates for enalaprilat, not less than 300 theoretical plates for enalapril, and not less than 2500 theoretical plates for enalapril diketopiperazine; the tailing factor for enalapril is not more than 2.0; the resolution, *R,* between maleic acid and enalaprilat is not less than 2.0, between enalaprilat and enalapril is not less than 2.0, and between enalapril and enalapril diketopiperazine is not less than 2.0. Chromatograph the *Standard preparation* as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0% for the enalapril peak, and responses for the enalaprilat peak agree within 5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in each T ablet taken by the formula:

$$(CV/N)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Enalapril Maleate RS in the *Standard preparation; V* is the nominal capacity, in mL, of the volumetric flask containing the *Assay preparation; N* is the number of T ablets taken for the *Assay preparation;* and r_U and r_S are the enalapril peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Enalapril Maleate and Hydrochlorothiazide Tablets

» Enalapril Maleate and Hydrochlorothiazide T ablets contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amounts of Enalapril Maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) and Hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Enalaprilat RS
USP Enalapril Maleate RS
USP Hydrochlorothiazide RS

Identification—

A: 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 for enalapril maleate*.

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 for hydrochlorothiazide*.

Dissolution ⟨711⟩**—**

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ dissolved, using filtered portions of the solution under test and following the *Procedure for content uniformity of enalapril maleate* in the test for *Uniformity of dosage units,* making any necessar y volumetric adjustments, in comparison with a Standard solution of USP Enalapril Maleate RS having similar concentrations in the same *Medium*.

Determine the amount of $C_7H_8ClN_3O_4S_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 320 nm and at 360 nm in 1-cm cells, on filtered portions of the solution under test, suitably diluted with *Medium,* in comparison with a Standard solution having a known concentration of USP Hydrochlorothiazide RS dissolved in 20 mL of methanol and diluted with *Medium*. Calculate the quantity, in mg, of hydrochlorothiazide dissolved by the formula:

$$(TC/D)(A_{320} - A_{360})_U / (A_{320} - A_{360})_S$$

in which *T* is the T ablet label claim, in mg, for hydrochlorothiazide; *C* is the concentration, in mg per mL, of hydrochlorothiazide in the Standard solution; *D* is the concentration, in mg per mL, of hydrochlorothiazide in the solution under test; and $(A_{320} - A_{360})_U$ and $(A_{320} - A_{360})_S$ are the differences in the absorbances at 320 and 360 nm of the solution under test and the Standard solution, respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) and not less than 60%