the relative standard deviation, determined from the valsartan related compound B peaks, for replicate injections is not more than 10.0%; and the relative standard deviation, determined from the valsartan peaks, for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of valsartan related compound B and valsartan related compound C in the portion of Valsartan taken by the formula: 

\[
100\left(\frac{C_B}{C_V} \times \frac{r_V}{r_B}\right)
\]

in which \(C_V\) is the concentration, in mg per mL, of the appropriate USP Valsartan Related Compound RS in the *Standard solution*; \(C_B\) is the concentration, in mg per mL, of valsartan in the *Test solution*; \(r_V\) is the peak response for the impurity obtained from the *Test solution*; and \(r_B\) is the peak response for the appropriate valsartan related compound obtained from the *Standard solution*. Calculate the percentage of each other impurity in the portion of Valsartan taken by the formula:

\[
100\left(\frac{C_I}{C_V} \times \frac{r_V}{r_I}\right)
\]

in which \(C_I\) is the concentration, in mg per mL, of the appropriate USP Valsartan Related Compound RS in the *Standard solution*; \(C_V\) is the concentration, in mg per mL, of valsartan RS in the *Standard solution*; \(r_I\) is the peak response for the impurity obtained from the *Standard solution*; \(r_V\) is the peak response for valsartan obtained from the *Standard solution*; and the other terms are as defined above: not more than 0.2% of valsartan related compound B is found; not more than 0.1% of valsartan related compound C is found; not more than 0.1% of any other individual impurity, excluding valsartan related compound A, is found; and not more than 0.3% of total impurities, excluding valsartan related compound A, is found.

**Assay**—

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (500:500:1). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Valsartan RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Transfer about 50 mg of Valsartan, 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 273-nm detector and a 3.0-mm × 12.5-cm column that contains 5-µm packing L1. The flow rate is about 0.4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of \(\text{C}_2\text{H}_{12}\text{N}_3\text{O}_3\) in the portion of Valsartan taken by the formula:

\[
100\left(\frac{C}{C_V} \times \frac{r_V}{r}\right)
\]

in which \(C\) is the concentration, in mg per mL, of USP Valsartan RS in the *Standard preparation*; and \(r\) and \(r_V\) are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Valsartan and Hydrochlorothiazide Tablets

**DEFINITION**

Valsartan and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of valsartan \((\text{C}_9\text{H}_{18}\text{N}_3\text{O}_3)\) and hydrochlorothiazide \((\text{C}_7\text{H}_5\text{Cl}_2\text{N}_2\text{O}_5\text{S}_2)\).

**IDENTIFICATION**

- **A. Thin-Layer Chromatographic Identification Test (201)**

  **Sample solution**: To an amount of ground Tablets, equivalent in weight to a single Tablet, add 2.0 mL of acetone, sonicate for 15 min, and centrifuge.

  **Application volume**: 2 µL.

  **Developing solvent system**: Ethyl acetate, dehydrated alcohol, and 3.6 M ammonium hydroxide (8:2:1).

  **Analysis**: Proceed as directed in the chapter, except to develop the plate in a paper-lined chromatographic chamber equilibrated with *Developing solvent system* for 15 min before use. Allow the chromatogram to develop until the solvent front has moved at least 7 cm. After removing the plate and marking the solvent front, dry the plate under a current of warm air. The \(R_f\) values of the principal spots obtained from the *Sample solution* correspond to those from the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **Procedure**

  **Diluent**: Acetonitrile and water (1:1)

  **Solution A**: Acetonitrile, water, and trtifluoroacetic acid (10:90:0.1)

  **Solution B**: Acetonitrile, water, and trifluoroacetic acid (90:10:0.1)

  **Mobile phase**: See the gradient table below.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>27</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>40</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

**Standard solution**: Transfer 12.5 mg of USP Hydrochlorothiazide RS to a 200-mL volumetric flask, and add 12.5J mg of USP Valsartan RS, to obtain a solution having a concentration of 0.2 mg/mL of USP Valsartan RS in *Diluent*.

**Sample stock solution**: To the equivalent of 62.5 mg of hydrochlorothiazide from a number of Tablets add 5 mL of water, and allow to stand for 5 min. Then add 100 mL of *Diluent*, sonicate for 15 min, and shake for 30 min. Dilute with *Diluent* to 250 mL, and centrifuge a portion of this solution at 3000 rpm. Dilute 25.0 mL of the clear supernatant with *Diluent* to 200.0 mL.

**Sample solution**: To the equivalent of 62.5 mg of hydrochlorothiazide from a number of Tablets add 5 mL of water, and allow to stand for 5 min. Then add 100 mL of *Diluent*, sonicate for 15 min, and shake for 30 min. Dilute with *Diluent* to 250 mL, and centrifuge a portion of this solution at 3000 rpm. Dilute 25.0 mL of the clear supernatant with *Diluent* to 200.0 mL.

**Chromatographic system**

*(See Chromatography (621), System Suitability.)*
UNIFORMITY OF DOSAGE UNITS

DISSOLUTION

Performance tests

Sample solutions: Official Monographs / USP = concentration of valsartan in the
Tolerances: Pass a portion of the solution under test
Sample solutions: USP Hydrochlorothiazide RS and USP
Standard solution:
Cell path length: 250 nm for valsartan and 272 nm hydrochlorothiazide,
Apparatus 2: 30 rpm compound B and valsartan and NL T 1.4 between
Medium: pH 6.8 phosphate buffer; 1000 mL

Acceptance criteria: NLT 80% (Q) of the labeled amounts of

IMPURITIES

Organic Impurities

Analysis

Samples: Standard solution and Sample solution
Calculate the percentage of C24H29N5O3 dissolved:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response from the Sample solution}
\]

\[
r_S = \text{peak response from the Standard solution}
\]

\[
C_S = \text{concentration of the appropriate USP Reference Standard in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of the corresponding analyte in the Sample solution (mg/mL)}
\]

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

Packaging and Storage: Preserve in tight containers, and
protect from moisture and heat. Store at controlled room temperature.

Additional requirements

Mode: LC
Detector: UV 265 nm
Column: 3.0-mm × 12.5-cm; 5-µm packing L1
Flow rate: 0.4 mL/min
Injection size: 10 µL

System suitability
Sample: Standard solution
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of C24H29N5O3 and C7H8ClN3O4S2 in the portion of Tablets taken:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response from the Sample solution}
\]

\[
r_S = \text{peak response from the Standard solution}
\]

\[
C_S = \text{concentration of the appropriate USP Reference Standard in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of the corresponding analyte in the Sample solution (mg/mL)}
\]

Acceptance criteria: Meet the requirements

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of each other impurity in the portion of Tablets taken:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response of benzothiadiazine related compound A from the Sample solution}
\]

\[
r_S = \text{peak response of benzothiadiazine related compound A from the Standard solution}
\]

\[
C_S = \text{concentration of benzothiadiazine related compound A in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of hydrochlorothiazide in the Sample solution (mg/mL)}
\]

Calculate the percentage of each other impurity in the portion of Tablets taken:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response of each other impurity from the Sample solution}
\]

\[
r_S = \text{peak response of valsartan from the Standard solution}
\]

\[
C_S = \text{concentration of valsartan in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of valsartan (for calculating other impurities) in the Sample solution (mg/mL)}
\]

Acceptance criteria: NMT 1.0% of benzothiadiazine related compound A; NMT 0.2% of any other impurity, excluding valsartan related compound A; NMT 1.3% of total impurities, excluding valsartan related compound A. [NOTE—Valsartan related compound A is the enantiomer of valsartan and coelutes with valsartan in this test.]

System suitability
Samples: System suitability solution and Standard solution
Suitability requirements
Resolution: NLT 80% (Q) of the labeled amounts of C24H29N5O3 and C7H8ClN3O4S2 is dissolved.

Uniformity of Dosage Units (905)

Procedure for content uniformity
Sample solution: Transfer 1 Tablet to a 200-mL volumetric flask, add 5 mL of water, and allow to stand for 5 min. Add 100 mL of Diluent, and sonicate for 15 min. Dilute with Diluent to 250 mL, mix, and centrifuge a portion of this solution at 3000 rpm. Dilute a volume of the clear supernatant with Diluent to obtain a solution having a concentration of 0.2 mg/mL of valsartan.

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of valsartan (C24H29N5O3) and hydrochlorothiazide (C7H8ClN3O4S2) in the Tablet taken:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response from the Sample solution}
\]

\[
r_S = \text{peak response from the Standard solution}
\]

\[
C_S = \text{concentration of the appropriate USP Reference Standard in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of the corresponding analyte in the Sample solution (mg/mL)}
\]

Acceptance criteria: Meet the requirements

Chromatographic system:
Result = \((\text{AT}_1 \times D) - (\text{AT}_2 \times B)\) / \((\text{D} - (\text{E} - \text{B}))\) × 80,000

\[\text{AT}_1 = \text{absorbance of the Sample solution at 272 nm}\]

\[\text{AT}_2 = \text{absorbance of the Sample solution at 250 nm}\]

\[B = \text{A1%} \times \text{V}_{272}, \text{absorbivity (1%, 0.2 cm, 272 nm) of valsartan in Medium}\]

\[C = \text{A1%} \times \text{V}_{250}, \text{absorbivity (1%, 0.2 cm, 250 nm) of valsartan in Medium}\]

\[D = \text{A1%} \times \text{V}_{272}, \text{absorbivity (1%, 0.2 cm, 272 nm) of hydrochlorothiazide in Medium}\]

\[E = \text{A1%} \times \text{V}_{250}, \text{absorbivity (1%, 0.2 cm, 250 nm) of hydrochlorothiazide in Medium}\]

Tolerances: NLT 80% (Q) of the labeled amounts of C24H29N5O3 and C7H8ClN3O4S2 is dissolved.

Packaging and Storage: Preserve in tight containers, and
protect from moisture and heat. Store at controlled room temperature.

Mode: LC
Detector: UV 265 nm
Column: 3.0-mm × 12.5-cm; 5-µm packing L1
Flow rate: 0.4 mL/min
Injection size: 10 µL

System suitability
Sample: Standard solution
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of valsartan (C24H29N5O3) and hydrochlorothiazide (C7H8ClN3O4S2) in the Tablet taken:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response from the Sample solution}
\]

\[
r_S = \text{peak response from the Standard solution}
\]

\[
C_S = \text{concentration of the appropriate USP Reference Standard in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of the corresponding analyte in the Sample solution (mg/mL)}
\]

Acceptance criteria: Meet the requirements

Analysis
Samples: Standard solution and Sample solution
Disregard the peak, if any, with a retention time of 22 min.
Calculate the percentage of benzenothiadiazine related compound A in the portion of Tablets taken:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response of benzothiadiazine related compound A from the Sample solution}
\]

\[
r_S = \text{peak response of benzothiadiazine related compound A from the Standard solution}
\]

\[
C_S = \text{concentration of benzothiadiazine related compound A in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of hydrochlorothiazide in the Sample solution (mg/mL)}
\]

Calculate the percentage of each other impurity in the portion of Tablets taken:

\[
\text{Result} = \frac{(r_U/r_S) \times (C_S/C_U) \times 100}{100}
\]

\[
r_U = \text{peak response of each other impurity from the Sample solution}
\]

\[
r_S = \text{peak response of valsartan from the Standard solution}
\]

\[
C_S = \text{concentration of valsartan in the Standard solution (mg/mL)}
\]

\[
C_U = \text{nominal concentration of valsartan (for calculating other impurities) in the Sample solution (mg/mL)}
\]

Acceptance criteria: NMT 1.0% of benzenothiadiazine related compound A; NMT 0.2% of any other impurity, excluding valsartan related compound A; NMT 1.3% of total impurities, excluding valsartan related compound A. [NOTE—Valsartan related compound A is the enantiomer of valsartan and coelutes with valsartan in this test.]
Vancomycin

\[ C_{66}H_{75}Cl_{2}N_{9}O_{24} \]

1449.25

Vancomycin

\[(S)_\{35,68,7,8,223,235,265,368,38a\}^{\alpha}((-3-Amino-2,3,6-trideoxy-3-C-methyl-a-L-lyxo-hexopyranosyl)-\beta-D-glucopyranosyl\}_2\{3-(carbamoylmethyl)-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38a-\text{tetradecahydro-7,22,28,30,32-pentahydroxy-6-[(2R)-4-methyl-2-(methylamino)valeronamido]-2,5,24,38,39-pentaoxaoctadecahydro-11,18,21-dietheno-23,36-(iminomethano)-13,16,31,35-dimetheno-1H,16H[1,6,9]\text{oxadiazacyclohexadecino[4,5-m][10,2,16]benzooxadiazacyclotetra-cosine-26-carboxylic acid;}

\[35-38,63,7,8,223,235,265,368,38a\}^{\alpha}((-3-Amino-2,3,6-trideoxy-3-C-methyl-a-L-lyxo-hexopyranosyl)-\beta-D-glucopyranosyl\}_2\{3-(carbamoylmethyl)-10,19-dichloro-2,3,4,5,6,7,23,24,25,26,36,37,38a-\text{tetradecahydro-7,22,28,30,32-pentahydroxy-6-[(2R)-4-methyl-2-(methylamino)valeronamido]-2,5,24,38,39-pentaoxaoctadecahydro-11,18,21-dietheno-23,36-(iminomethano)-13,16,31,35-dimetheno-1H,16H[1,6,9]\text{oxadiazacyclohexadecino[4,5-m][10,2,16]benzooxadiazacyclotetra-cosine-26-carboxylic acid;}

Vancomycin has a potency equivalent to NLT 950 \( \mu \text{g} \)/mg of \( C_{66}H_{75}Cl_{2}N_{9}O_{24} \), calculated on the anhydrous basis.

**IDENTIFICATION**

A. Infrared Absorption (197K)

B. Identification Tests—General, Chloride (191): It does not meet the requirements of the test.

**ASSAY**

Antibiotics—Microbial Assays (81)

Sample solution: Transfer 250 mg of Vancomycin to a 25-mL volumetric flask. Add 5 mL of Solution A, then add 0.1 N hydrochloric acid dropwise with swirling until dissolution is achieved. Dilute with Solution A to volume.

Sample solution: Dilute 2.0 mL of the Sample solution to 20 mL with Solution A.

Chromatographic system

See Chromatography (621), System Suitability.

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \( \times \) 25-cm; 5-\( \mu \)m packing L1

Flow rate: 20 \( \mu \)L/min

Injection size: 20 \( \mu \)L

System suitability

Sample: System suitability solution

[Note—The elution order is compound 1, vancomycin B, and compound 2. Compound 2 elutes 3–6 min after the start of the period when the percentage of Solution B is increasing from 0% to 100%.]

Suitability requirements

Resolution: NLT 3.0 between compound 1 and vancomycin B

Column efficiency: NLT 1500 theoretical plates for the vancomycin B peak

Analysis

Samples: Sample stock solution and Sample solution

Where baseline separation is not achieved, peak areas are defined by vertical lines extended from the valleys between peaks to the baseline. The main component peak may include a fronting shoulder, which is attributed to monodechlorovancomycin. This shoulder should not be integrated separately.

Correct any peak observed in the chromatograms obtained from the Sample stock solution and Sample solution by subtracting the area response of any peak observed in the chromatogram of Solution A at the corresponding retention time.

Calculate the percentage of vancomycin B in the portion of Vancomycin taken:

\[ \text{Result} = \left( \frac{D \times n_A}{(D \times n_B) + n_A} \right) \times 100 \]

\[ D = \text{dilution factor, Sample stock solution to Sample solution, 25} \]

\[ n_B = \text{corrected peak area response of the main peak from the Sample solution} \]

\[ n_A = \text{sum of the corrected peak area responses of all the peaks, other than the main peak, from the Sample stock solution} \]

Calculate the percentage of any individual peak, other than the main peak, in the portion of Vancomycin taken:

\[ \text{Result} = \left( \frac{r_i}{(D \times n_B) + n_A} \right) \times 100 \]

\[ r_i = \text{corrected peak area response of any individual peak, other than the main peak, from the Sample stock solution} \]

\[ D = \text{dilution factor, Sample stock solution to Sample solution, 25} \]

\[ r_B = \text{corrected peak area response of the main peak from the Sample solution} \]