Procedure—Separately inject equal volumes (about 5 µL) of the Standard preparation and the Test preparation into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the weight, in mg, of the major peaks. Calculate the weight, in mg, of isopropyl alcohol in the portion of Warfarin Sodium taken by the formula:

\[
100C(R_o / R_i)
\]

in which \(C\) is the concentration, in mg per mL, of isopropyl alcohol in the Standard preparation; and \(R_o\) and \(R_i\) are the peak area ratios of isopropyl alcohol to \(n\)-propyl alcohol obtained from the Test preparation and the Standard preparation, respectively.

Assay—

**pH 7.4 Buffer**—Transfer 1.36 g of monobasic potassium phosphate to a 200-mL volumetric flask, and dissolve in 50 mL of water. Add 39.1 mL of 0.2 N sodium hydroxide, and dilute with water to volume. Adjust with sodium hydroxide or phosphoric acid to a pH of 7.4 ± 0.1.

**Mobile phase**—Prepare a degassed solution containing a mixture of methanol, water, and glacial acetic acid (64:36:1). Adjust the ratio as necessary.

**Standard preparation**—Transfer about 94 mg of USP Warfarin RS, accurately weighed, to a 250-mL volumetric flask, and dissolve in 97.8 mL of 0.1 N sodium hydroxide. Add 62.5 mL of 0.2 M monobasic potassium phosphate, dilute with water to volume, and mix. Pipet 5 mL of this solution and 15 mL of pH 7.4 Buffer into a conical flask, and mix.

**Assay preparation**—Using about 100 mg of Warfarin Sodium, accurately weighed, prepare as directed for Standard preparation.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.4 mL per minute. Chromatograph five replicate injections of the Standard preparation, and record the peak responses as directed for Procedure: the relative standard deviation of the warfarin responses 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 the responses for the major peaks. Calculate the quantity, in mg, of C\(_{19}\)H\(_{15}\)NaO\(_{4}\) in the portion of Warfarin Sodium taken by the formula:

\[
(330.31 / 308.34)C(r_o / r_i)
\]

in which 330.31 and 308.34 are the molecular weights of warfarin sodium and warfarin, respectively; \(C\) is the concentration, in µg per mL, of USP Warfarin RS in the Standard preparation; and \(r_o\) and \(r_i\) are the peak responses of warfarin obtained from the Assay preparation and the Standard preparation, respectively.

**Completeness of solution** (641)—A 1.0-g portion dissolves in 10 mL of carbon dioxide-free water to yield a clear solution.

**Constitutioned solution**—At the time of use, it meets the requirements for Constitutioned Solutions under Injections (1).

**Bacterial endotoxins** (85)—It contains not more than 24.0 USP Endotoxin Units per mg of warfarin sodium.

**Water, Method I** (921): not more than 4.5%.

**Other requirements**—It responds to Identification tests A and B, and meets the requirements for pH and Heavy metals under Warfarin Sodium. It meets also the requirements for Sterility Tests (71), Uniformity of Dosage Units (905), and Labeling under Injections (1).

**Assay**—

**pH 7.4 Buffer, Mobile phase, and Chromatographic system**—Prepare as directed in the Assay under Warfarin Sodium.

**Standard preparation**—Transfer about 94 mg of USP Warfarin RS, accurately weighed, to a 100-mL volumetric flask, and dissolve in 39.1 mL of 0.1 N sodium hydroxide. Add 25.0 mL of 0.2 M monobasic potassium phosphate, dilute with water to volume, and mix. Pipet 5 mL of this solution into a 50-mL volumetric flask, dilute with pH 7.4 Buffer to volume, and mix.

**Assay preparation**—Dissolve the contents of not fewer than 10 containers of Warfarin Sodium for Injection in a sufficient volume, accurately measured, of pH 7.4 Buffer to obtain a solution containing about 1 mg of warfarin sodium per mL. Pipet 5 mL of the resulting solution into a 50-mL volumetric flask, dilute with pH 7.4 Buffer to volume, and mix.

**Procedure**—Proceed as directed for Procedure in the Assay under Warfarin Sodium. Calculate the average quantity, in mg, of warfarin sodium (C\(_{19}\)H\(_{15}\)NaO\(_{4}\)) in each container of Warfarin Sodium for Injection taken by the formula:

\[
10(330.31 / 308.34)VC(N(r_o / r_i))
\]

in which 330.31 and 308.34 are the molecular weights of warfarin sodium and warfarin, respectively; \(V\) is the volume, in mL, of the solution prepared from the contents of the 10 or more containers; \(C\) is the concentration, in mg per mL, of USP Warfarin RS in the Standard preparation; \(N\) is the number of containers taken; and \(r_o\) and \(r_i\) are the peak responses of warfarin obtained from the Assay preparation and the Standard preparation, respectively.

**Warfarin Sodium Tablets**

» Warfarin Sodium Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of warfarin sodium (C\(_{19}\)H\(_{15}\)NaO\(_{4}\)).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Warfarin RS

**Identification**—

A: The retention time of the major peak obtained from the Assay preparation corresponds to that obtained from the Standard preparation as directed in the Assay.

B: Infrared Absorption (197K)—Prepare the test specimen as follows. Triturate a quantity of finely powdered Tablets, equivalent to about 200 mg of warfarin sodium, with 50 mL of water, centrifuge, and filter the supernatant. Extract with 50 mL of ether, transfer the aqueous layer to a second separator, and discard the ether. Adjust with hydrochloric acid to a pH of less than 3, using short-range pH indicator paper, and extract with 50 mL of chloroform. Transfer the chloroform layer to another separator, extract with 50 mL of sodium hydroxide solution (1 in 250), and discard the chloroform. Transfer the aqueous layer
to a beaker, and adjust with hydrochloric acid to a pH of less than 3 (using the pH indicator paper) to precipitate the warfarin. Stir the mixture and allow the precipitate to coagulate. Filter, and wash the precipitate with four 5-mL portions of water. If the precipitate is not white or practically white, dissolve it in a minimum volume of sodium hydroxide solution (1 in 250), dilute with water to 50 mL, and repeat the foregoing procedure, beginning with “Extract with 50 mL of ether.” Dry the warfarin so obtained in vacuum over phosphorus pentoxide for 4 hours.

**Dissolution (711)—**

<table>
<thead>
<tr>
<th>Medium:</th>
<th>water; 900 mL.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparatus 2:</td>
<td>50 rpm.</td>
</tr>
<tr>
<td>Time:</td>
<td>30 minutes.</td>
</tr>
</tbody>
</table>

**Mobile phase and Chromatographic system—Proceed as directed in the Assay.**

**Standard solution**—Dissolve an accurately weighed quantity of USP Warfarin RS in water to obtain a solution having a known concentration of about 0.0008 L mg per mL. Let the labeled amount, in mg, of warfarin sodium in the Tablets. [NOTE—Use a small amount of 0.1 N sodium hydroxide to aid in dissolution.]

**Procedure**—Separately inject equal volumes (about 40 µL) of the Standard solution and a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of warfarin sodium dissolved by the formula:

\[ \frac{(330.31 \times 308.34)(9000)}{C} \left( \frac{r_0}{r_s} \right) \]

in which 330.31 and 308.34 are the molecular weights of warfarin sodium and warfarin, respectively; \( C \) is the concentration, in mg per mL of USP Warfarin RS in the Standard solution; and \( r_0 \) and \( r_s \) are the peak responses of warfarin obtained from the solution under test and the Standard solution, respectively.

**Uniformity of dosage units (905):** meet the requirements.

**Assay—**

pH 7.4 Buffer and Chromatographic system—Proceed as directed in the Assay under Warfarin Sodium.

**Solvent mixture**—Prepare a mixture of pH 7.4 Buffer and acetonitrile (85:15).

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (68:32:1). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard preparation**—Transfer about 62.5 mg of USP Warfarin RS, accurately weighed, to a 200-mL volumetric flask, and dissolve in 78 mL of 0.1 N sodium hydroxide. Add 50 mL of 0.2 M monobasic potassium phosphate, dilute with water to volume, and mix. Transfer 15.0 mL of this solution to a 50-mL volumetric flask, and dilute with Solvent mixture to volume.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of warfarin sodium, to a 50-mL volumetric flask, and add about 30 mL of Solvent mixture. Sonicate for 10 minutes, and then shake by mechanical means for 60 minutes. Dilute with Solvent mixture to volume, and filter.

**Procedure**—Separately inject equal volumes (about 20 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 C\(_{19}\)H\(_{15}\)NaO\(_{4}\) in the portion of Tablets taken by the formula:

\[ \frac{50(330.31 \times 308.34)}{C} \left( \frac{r_0}{r_s} \right) \]

in which 330.31 and 308.34 are the molecular weights of warfarin sodium and warfarin, respectively; \( C \) is the concentration, in mg per mL of USP Warfarin RS in the Standard preparation; and \( r_0 \) and \( r_s \) are the peak responses of warfarin obtained from the Assay preparation and the Standard preparation, respectively.

**Water for Hemodialysis**

[NOTE—See Water for Hemodialysis Applications (1230) for guidelines on microbial and chemical testing.]

**DEFINITION**

Water for Hemodialysis is water that complies with the U.S. Environmental Protection Agency National Primary Drinking Water Regulations and that has been subjected to further treatment, using a suitable process, to reduce chemical and microbiological components. It is produced and used onsite under the direction of qualified personnel. It contains no added antimicrobials and is not intended for injection.

**SPECIFIC TESTS**

- **Total Organic Carbon (643):** Meets the requirements
- **Microbial Enumeration Tests (61) and Tests for Specified Microorganisms (62):** The total aerobic microbial count does not exceed 100 cfu/mL. It meets the requirements of the test for absence of *Pseudomonas aeruginosa*.
- **Water Conductivity, Bulk Water (645):** Meets the requirements
- **Bacterial Endotoxins Test (85):** It contains less than 1 USP Endotoxin Unit/mL.

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in unreactive storage containers that are designed to prevent bacterial entry. Store at room temperature.
- **USP Reference Standards (11)***
  - USP 1,4-Benzquinone RS
  - USP Endotoxin RS
  - USP Sucrose RS

**Water for Injection**

[NOTE—For microbiological guidance, see general information chapter Water for Pharmaceutical Purposes (1231).]

**DEFINITION**

Water for Injection is water purified by distillation or a purification process that is equivalent or superior to distillation in the removal of chemicals and microorganisms. It is prepared from water complying with the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or with the drinking water regulations of the European Union or of Japan or with the World Health Organization’s Guidelines for Drinking Water Quality. It contains no added substance.

[NOTE—Water for Injection, whether it is available in bulk or packaged forms, is intended for use in the preparation of parenteral solutions. Where used for the preparation of parenteral solutions subject to final sterilization, use suitable means to minimize microbial growth, or first render the Water for Injection sterile and, thereafter, protect it from microbial contamination. For parenteral solutions that are prepared under aseptic conditions and are not sterilized by appropriate filtration or in the final container, first render the Water for Injection sterile and, thereafter, protect it from microbial contamination. In addition to the Specific Tests, Water for Injection that is packaged for commercial use elsewhere meets the additional requirements for Packaging and Storage and Labeling as indicated under Additional Requirements.]