solution. Calculate the percentage of each other impurity by the formula:

$$0.2C_{\text{av}}(r_{\text{i}} / r_{\text{Sm}})$$

in which $C_{\text{av}}$ is the concentration, in $\mu g$ per mL, of USP Mesalamine RS in the Standard solution; $r_{\text{i}}$ is the response of the individual impurity peak in the chromatogram obtained from the Test solution; and $r_{\text{Sm}}$ is the response of the mesalamine peak in the chromatogram obtained from the Standard solution: not more than 0.2% of 3-aminosalicylic acid is found; not more than 0.2% of any other impurity, expressed in terms of mesalamine equivalent, is found; and the total of all impurities found is not more than 1.0%.

**TEST 2 (for aniline, 2-aminophenol, and 4-aminophenol)—**

**Standard solution**—Prepare a solution of aniline, 2-aminophenol, and 4-aminophenol in methanol having concentrations of 0.05, 2, and 2 $\mu g$ per mL, respectively; and dilute quantitatively, and stepwise if necessary, with methylene chloride to obtain a solution having concentrations of 0.5, 20, and 20 $\mu g$ per mL, respectively.

**Test solution**—Mix 1.0 g of Mesalamine with 10.0 mL of methylene chloride. Allow to settle, and use the clear methylene chloride solution as the Test solution.

**Chromatographic system (see Chromatography (621))**—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm x 10-m fused-silica capillary column coated with a 2.65-µm film of stationary phase G27. The carrier gas is helium flowing at a rate of 15 mL per minute. The injection port and the detector temperatures are maintained at about 280° and 300°, respectively. The column temperature is programmed according to the following steps: the starting column temperature is 70°; after injection it is held at 70° for 2 minutes, then increased to 150° at a rate of 30° per minute, then held for 1 minute. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.5 for aniline, 0.9 for 2-aminophenol, and 1.0 for 4-aminophenol; and the peaks are baseline separated.

**Procedure**—Separately inject equal volumes (about 2 $\mu L$) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak area responses. Identify by retention time any peaks present in the chromatogram of the Test solution that correspond to those in the chromatogram obtained from the Standard solution. Calculate the quantities, in $\mu g$ per g, of aniline, 2-aminophenol, and 4-aminophenol in the portion of Mesalamine taken by the formula:

$$10C(r_{\text{i}} / r_{\text{Sm}})$$

in which $C$ is the concentration, in $\mu g$ per mL, of the relevant analyte in the Standard solution; $r_{\text{i}}$ is the response of the relevant analyte in the chromatogram obtained from the Test solution; and $r_{\text{Sm}}$ is the response of the relevant analyte in the chromatogram obtained from the Standard solution: not more than 5 $\mu g$ of aniline, 200 $\mu g$ of 2-aminophenol, and 200 $\mu g$ of 4-aminophenol per g are found.

**Assay**—

**Buffer solution**—Transfer 7.1 g of anhydrous dibasic sodium phosphate and 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 7.5 mL of a solution of tetrabutylammonium hydroxide in methanol (1 in 4), dilute with water to volume, and mix.

**Mobile phase**—Prepare a suitable degassed mixture of Buffer solution and methanol (85:15). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Resolution solution**—Prepare a solution in Mobile phase containing about 0.25 mg of 4-aminosalicylic acid and 0.4 mg of USP Mesalamine RS per mL.

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Mesalamine RS in Mobile phase to obtain a solution having a known concentration of about 1 mg per mL. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with Mobile phase to volume, and mix. This solution contains about 0.4 mg of USP Mesalamine RS per mL.

**Assay preparation**—Transfer about 50 mg of Mesalamine, accurately weighed, to a 50-mL volumetric flask, dilute in and dilute with Mobile phase to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with Mobile phase to volume, and mix.

**Chromatographic system (see Chromatography (621))**—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm x 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the resolution, $R$, between 4-aminosalicylic acid and mesalamine is not less than 2.0. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 15 $\mu L$) 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$_7$H$_7$NO$_3$ in the portion of Mesalamine taken by the formula:

$$125C(r_{\text{i}} / r_{\text{Sm}})$$

in which $C$ is the concentration, in mg per mL, of USP Mesalamine RS in the Standard preparation; and $r_{\text{i}}$ and $r_{\text{Sm}}$ are the responses of the mesalamine peaks obtained from the Assay preparation and the Standard preparation, respectively.

**Mesalamine Extended-Release Capsules**

> Mesalamine Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mesalamine (C$_7$H$_7$NO$_3$).

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

**USP Reference standards** (11)—

USP Mesalamine RS

**Identification, Infrared Absorption (197K);** the powdered, undried Capsule contents being used, and the spectra being recorded in the range between 2000 cm$^{-1}$ and 1240 cm$^{-1}$.

**Dissolution (711)—**

Medium: 0.05 M pH 7.5 phosphate buffer prepared by dissolving 6.8 g of monobasic potassium phosphate and 1 g of sodium hydroxide in water to make 1000 mL of solution, and adjusting with 10 N sodium hydroxide to a pH of 7.50 ± 0.05; 900 mL.

Apparatus 2: 100 rpm.

Times: 1, 2, 4, and 8 hours.

**Procedure**—Determine the amount of C$_7$H$_7$NO$_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 330 nm on filtered portions of the solution under test suitably diluted with Medium, if necessary, in comparison with a Standard solution having a known concentration of USP Mesalamine RS in the same Medium.

**Tolerances**—The percentages of the labeled amount of C$_7$H$_7$NO$_3$ dissolved at the times specified conform to Acceptance Table 2.
Uniformity of dosage units (905): meet the requirements.

Assay—

Buffer—Dissolve 6.8 g of monobasic potassium phosphate and 1.65 g of sodium hydroxide in 800 mL of water, adjust with 1 N sodium hydroxide to a pH of 7.5, dilute with water to 1000 mL, and mix.

Mobile phase A—Dissolve 3.4 g of tetrabutylammonium hydrogen sulfate and 1.4 g of sodium acetate trihydrate in 1000 mL of water, and adjust with 1 N sodium hydroxide to a pH of 6.6. Add 200 mL of acetonitrile, mix, and pass through a filter having a 0.5-µm or finer porosity. Make any necessary adjustments (see System Suitability under Chromatography (621)).

NOTE—Increasing the proportion of acetonitrile decreases the retention times. Prepare fresh daily.

Mobile phase B—Dissolve 4.6 g of tetrabutylammonium hydrogen sulfate and 1.9 g of sodium acetate trihydrate in 1000 mL of water, and adjust with 1 N sodium hydroxide to a pH of 6.6. Add 650 mL of acetonitrile, mix, and pass through a filter having a 0.5-µm or finer porosity. Make any necessary adjustments (see System Suitability under Chromatography (621)).

NOTE—Prepare fresh daily.

Internal standard solution—Prepare a solution in Buffer containing about 35 mg of sodium benzoate per mL.

Standard preparation—Transfer about 50 mg of USP Mesalamine RS, accurately weighed, to a 100-mL volumetric flask, add 4.0 mL of Internal standard solution, mix, dilute with Buffer to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with Buffer to volume, and mix.

Assay preparation—Transfer, as completely as possible, the contents of not fewer than 20 Capsules to a suitable tared container, and determine the average weight of the contents of a Capsule. Finely powder the Capsule contents so that the powder thus obtained passes through a No. 40 sieve (see Powder Fineness (811)). Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of mesalamine, to a 500-mL volumetric flask, add 20.0 mL of Internal standard solution, and about 300 mL of Buffer, and shake by mechanical means for 1 hour. Dilute with Buffer to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with Buffer to volume, mix, and pass about 10 mL of this solution through a filter having a 0.5-µm or finer porosity. Use the filtrate as the Assay preparation.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1, and is programmed to provide variable mixtures of Mobile phase A and Mobile phase B. The flow rate is about 1.5 mL per minute. The system is equilibrated with Mobile phase A. Five minutes after the injection of the Standard preparation and the Assay preparation, the proportion of Mobile phase B is increased linearly from 0% to 100% over a period of 2 minutes, and held for 8 minutes. The proportion of Mobile phase A is then increased linearly from 100% to 0% over a period of 2 minutes and held for 3 minutes. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative retention times are about 0.6 for mesalamine and 1.0 for sodium benzoate, the resolution, R, between mesalamine and sodium benzoate; is not less than 2.5; and 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 responses for the major peaks. Calculate the quantity, in mg, of mesalamine (C7H7NO3) in the portion of Capsule contents taken by the formula:

$$\frac{2500C}{V(r_U / r_S)}$$

in which C is the concentration, in mg per mL, of USP Mesalamine RS in the Standard preparation; and rU and rS are the peak response ratios of the mesalamine peak to the sodium benzoate peak obtained from the Assay preparation and the Standard preparation, respectively.

Mesalamine Rectal Suspension

MESALAMINE RECTAL SUSPENSION is a suspension of Mesalamine in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mesalamine (C7H7NO3). It contains one or more suitable preservatives.

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

USP Reference standards (11)—

USP Mesalamine RS

Identification—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.

Uniformity of dosage units (905): meets the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Buffer solution, Mobile phase, Resolution solution, and Chromatographic system—Proceed as directed in the Assay under Mesalamine.

Standard solution—Transfer about 100 mg of USP Mesalamine RS, accurately weighed, to a 50-mL volumetric flask, add 15 mL of 2 N hydrochloric acid, and dissolve by swirling. Dilute with 2 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with Buffer to volume, mix, and pass about 10 mL of this solution through a filter having a 0.5-µm or finer porosity.

Test solution—Transfer, with the aid of 2 N hydrochloric acid, the contents of a container of Rectal Suspension to a 200-mL volumetric flask. Add 2 N hydrochloric acid to obtain about 160 mL of solution, and shake for about 10 minutes. Dilute with 2 N hydrochloric acid to volume, and mix. Transfer an accurately measured volume of this stock solution, equivalent to about 40 mg of mesalamine, to a 100-mL volumetric flask, add 5 mL of 2 N sodium hydroxide, dilute with Mobile phase to volume, and mix. Pass this solution through a suitable filter having a 0.5-µm or finer porosity.

Procedure—Proceed as directed in the Assay. Calculate the quantity, in g, of C7H7NO3 in the container of Rectal Suspension taken by the formula:

$$20(C / V)(r_U / r_S)$$

in which V is the volume, in mL, of stock solution taken to prepare the Test solution; and the other terms are as defined therein.

pH (791): between 3.5 and 5.5, when diluted 1 to 10 with water.

Related compounds—

Mobile phase, Standard solution, and Chromatographic system—Proceed as directed in Test 1 for Related compounds under Mesalamine.