Test solution—Transfer an accurately measured volume of Rectal Suspension, previously well shaken, equivalent to 100 mg of mesalamine, to a beaker, add water to give a volume of about 80 mL, adjust with phosphoric acid to a pH of 2.0, sonicate briefly to dissolve, transfer to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed as directed in the test for Related compounds under Mesalamine. Calculate the percentage of each impurity in the Rectal Suspension taken by the formula:

\[0.1C_\text{a}(t_0 / t_1)\]

in which the terms are as defined therein. Not more than 0.2% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Content of sodium benzoate (if present)—

Mobile phase—Transfer 390 mg of ammonium acetate to a 1000-mL volumetric flask, add 100 mL of water, and dissolve by swirling. Add 6 mL of glacial acetic acid and 300 mL of methanol, dilute with water to volume, and mix. Pass this solution through a filter having a 0.5-μm or finer porosity. Make adjustments if necessary (see System Suitability under Chromatography (621)).

Standard solution—Transfer about 100 mg of sodium benzoate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Pass 5.0 mL of this solution to a second 100-mL volumetric flask, add 40 mL of methanol, dilute with water to volume, and mix. Pass this solution through a filter having a 0.5-μm or finer porosity.

Test solution—Transfer about 5 g of well-shaken Rectal Suspension, accurately weighed, to a 100-mL volumetric flask, add 40 mL of methanol, dilute with water to volume, and mix. Pass this solution through a filter having a 0.5-μm or finer porosity.

Chromatographic system—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Inject the Standard solution into the chromatograph, 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 μL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage (w/w) of sodium benzoate in the Rectal Suspension taken by the formula:

\[10(C/W)(t_0 / t_1)\]

in which C is the concentration, in mg per mL, of sodium benzoate in the Standard solution; W is the weight, in g, of the Rectal Suspension taken; and \(t_0\) and \(t_1\) are the responses obtained from the Test solution and the Standard solution, respectively: it contains between 0.05% and 0.125% of sodium benzoate.

Assay—

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

Assay preparation—Transfer an accurately measured, well-shaken quantity of Rectal Suspension, equivalent to about 0.5% of the labeled amount of mesalamine (C\(_7\)H\(_7\)NO\(_3\)) in the portion of Rectal Suspension taken by the formula:

\[250C(t_0 / t_1)\]

in which the terms are as defined therein.

Mesalamine Delayed-Release Tablets

Mesalamine Delayed-Release Tablets 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 containers.

USP Reference standards (11)—

USP Mesalamine RS
USP Salicylic Acid RS

Identification, Infrared Absorption (197K)—

Test specimen—To about 50 mL of water add a quantity of finely powdered Tablets, equivalent to about 800 mg of mesalamine. Boil the mixture for about 5 minutes, with constant stirring. Filter the hot solution, and allow the filtrate to cool. Collect the precipitated crystals, and dry at about 110°C.

Dissolution (711)—

pH 6.0 Phosphate buffer—Transfer about 43.35 g of monobasic potassium phosphate and 1.65 g of sodium hydroxide to a 2-L volumetric flask. Dissolve in and dilute with water to volume, and mix. Add 1 N sodium hydroxide or phosphoric acid to a pH of 6.0, and mix.

Sodium hydroxide solution—Transfer 133.6 g of sodium hydroxide to a 2-L volumetric flask, dissolve in and dilute with water to volume, and mix.

Medium: 0.1 N hydrochloric acid, 500 mL for Acid stage; pH 6.0 Phosphate buffer, 900 mL for Buffer stages.

Apparatus 2: 100 rpm for Acid stage and for Buffer stage 1; 50 rpm for Buffer stage 2.

Times: 2 hours for Acid stage; 1 hour for Buffer stage 1; 90 minutes for Buffer stage 2.

ACID STAGE—After 2 hours of operation, withdraw an aliquot of the fluid, discard the remaining solution, and retain the Tablets in proper order, so that each will be returned to its respective vessel later on. Blot the Tablets with a paper towel to dry, and proceed immediately as directed for Buffer stage 1.

Procedure—Determine the amount of C\(_7\)H\(_7\)NO\(_3\) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 250 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, equivalent to about 1% of the labeled amount of C\(_7\)H\(_7\)NO\(_3\) in the same Medium.

Tolerances—The percentage of the labeled amount of C\(_7\)H\(_7\)NO\(_3\) dissolved from the units tested conforms to the Acceptance Table shown below. Continue testing through all levels unless the results conform at an earlier level.

BUFFERS STAGE 1—[NOTE—Use buffer that has been equilibrated to a temperature of 37 ± 0.5°C] Transfer pH 6.0 Phosphate buffer to each of the dissolution vessels, and place each Tablet from the Acid stage into its respective vessel. After 1 hour remove a 50-mL aliquot, and proceed immediately as directed for Buffer stage 2.

Procedure—Determine the amount of C\(_7\)H\(_7\)NO\(_3\) dissolved by employing UV absorption 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, equivalent to about 1% of the labeled amount of C₂H₇NO₃, in the same Medium.

Tolerances—The percentage of the labeled amount of C₂H₇NO₃ dissolved from the units tested conforms to the Acceptance Table shown below. Continue testing through all levels unless the results conform at an earlier level.

<table>
<thead>
<tr>
<th>Level</th>
<th>Number Tested</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>L₁</td>
<td>6</td>
<td>No individual value exceeds 1% dissolved.</td>
</tr>
<tr>
<td>L₂</td>
<td>6</td>
<td>Average of the 12 units (L₁ + L₂) is not more than 1% dissolved, and no individual unit is greater than 10% dissolved.</td>
</tr>
<tr>
<td>L₃</td>
<td>12</td>
<td>Average of the 24 units (L₁ + L₂ + L₃) is not more than 1% dissolved, and not more than one individual unit is greater than 10% dissolved.</td>
</tr>
</tbody>
</table>

Acceptance Table

BUFF STAGE—Add 50 mL of Sodium hydroxide solution to each dissolution vessel to adjust to a pH of 7.2, and continue the run.

Procedure—Determine the amount of C₂H₇NO₃ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 332 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—Not less than 80% (Q) of the labeled amount of C₂H₇NO₃ is dissolved. The requirements are met if the quantities dissolved from the product conform to Acceptance Table 4. Continue testing through all levels unless the results conform at an earlier level.

Uniformity of dosage units (905): meet the requirements for Weight Variation.

Chromatographic purity—

Mobile phase—Proceed as directed in the Assay.

Chromatographic system—Proceed as directed in the Assay. To evaluate the system suitability requirements, use the System suitability preparation, Standard stock preparation, and the Standard preparation prepared as directed in the Assay.

Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 400 mg of mesalamine, to a 500-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, and sonicate to dissolve. Shake by mechanical means for 10 minutes, dilute with water to volume, and pass through a filter having a 0.5-μm or finer porosity. [NOTE—Use an aliquot of this solution for the Assay preparation.]

Procedure—Inject a volume (about 20 μL) of the Test solution into the chromatograph, record the chromatogram, and measure the areas for all the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

\[
100 \left( \frac{r_i}{r_s} \right)
\]

in which \( r_i \) is the peak response for each impurity; and \( r_s \) is the sum of the responses of all the peaks: the largest secondary peak is not more than 1.0% of the total area; not more than 0.5% of any other individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Dissolve 4.3 g of sodium 1-octanesulfonate in 1 L of water. Adjust with phosphoric acid to a pH of 2.15, pass through a filter having a 0.45-μm or finer porosity, and degas.

System suitability preparation—Transfer about 20 mg each of 3-aminosalicylic acid and USP Salicylic Acid RS, accurately weighed, to a 200-mL volumetric flask. Dissolve in 50 mL of 1 N hydrochloric acid, sonicking to dissolve, dilute with water to volume, and mix. Dilute the solution so obtained quantitatively and stepwise with water, and mix to obtain a solution having known concentrations of about 0.01 mg each of 3-aminosalicylic acid and salicylic acid per mL.

Standard stock preparation—Transfer about 25 mg of USP Mesalamine RS, accurately weighed, to a 25-mL volumetric flask. Dissolve in 5 mL of 0.25 N hydrochloric acid, sonicking to dissolve, dilute with water to volume, and mix.

Standard preparation—Transfer 10.0 mL of Standard stock preparation and 5.0 mL of System suitability preparation to a 50-mL volumetric flask. Dilute with water to volume, mix, and pass through a filter having a 0.5-μm or finer porosity.

Assay preparation—Pipet a 25.0-mL aliquot of the Test solution, obtained as directed for the Chromatographic purity test, into a 100-mL volumetric flask, dilute with water to volume, mix, and pass through a filter having a 0.5-μm or finer porosity.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 230-nm detector, a 4.6-mm × 3.3-cm analytical column that contains 3-μm base-deactivated packing L1, and two 4.6-mm × 3.0-cm precolumns, each containing 10-μm packing L1 and being located between the pump and the injector. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the resolution, R, between mesalamine and salicylic acid or 3-aminosalicylic acid is not less than 2; the tailing factor is not more than 2; 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 the responses for the major peaks. Calculate the quantity, in mg, of mesalamine (C₇H₇NO₃) in the portion of Tablets taken by the formula:

\[
2000C(r_o / r_s)
\]

in which C is the concentration, in mg per mL, of USP Mesalamine RS in the Standard preparation; and \( r_o \) and \( r_s \) are the mesalamine peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Mesna

C₇H₇NaO₃S₂ 164.18

Ethanesulfonic acid, 2-mercapto-, monosodium salt; Sodium 2-mercaptoethanesulfonate; Sodium 2-sulphonylethanesulfonate [19767-45-4].

DEFINITION
Mesna contains NLT 96.0% and NMT 102.0% of C₇H₇NaO₃S₂, calculated on the dried basis.

IDENTIFICATION
A. INFRARED ABSORPTION (197K)
B. IDENTIFICATION TESTS—GENERAL, Sodium (191): A solution meets the requirements of the flame test.

ASSAY
Procedure
Sample solution: 120 mg of Mesna in 10 mL of water
Analysis: To the Sample solution add 10 mL of 1 M sulfuric acid and 10 mL of 0.1 N iodine VS. Titrate with 0.1 N sodium thiosulfate VS, adding 1 mL of starch TS near the endpoint. Perform a blank determination, and make any necessary correction (see Titrimetry (541)). Each mL of sodium thiosulfate is equivalent to 16.42 mg of C₇H₇NaO₃S₂.