Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1
Column temperature: 50°
[NOTE—Preheat the *Mobile phase* to 50°.]
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for the two epimers of 11-ketobudesonide are 0.73 and 0.78, respectively; the relative retention times for 21-dehydrobudesonide, 14,15-dehydrobudesonide, and the first eluted epimer of budesonide (epimer B) are 0.68, 0.84, and 1.0, respectively.]
Suitability requirements
Column efficiency: NLT 5500 theoretical plates, determined from the budesonide epimer B peak
Analysis
Sample: *Sample solution*
Calculate the percentage of 11-ketobudesonide in the portion of Budesonide taken:

$$\text{Result} = (r_{T1}/r_{T2}) \times 100$$

r_{T1} = sum of the peak areas for the two ketobudesonide peaks
r_{T2} = sum of the peak areas of the two budesonide peaks

Acceptance criteria: NMT 0.2% of 11-ketobudesonide is found.

- **PROCEDURE 3**

Buffer: 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid. The pH is 3.2 ± 0.1.
Mobile phase: Acetonitrile and *Buffer* (32:68)
Standard solution: Dissolve a quantity of USP Budesonide RS in acetonitrile, and dilute quantitatively with *Buffer* to obtain a solution having a concentration of 0.5 mg/mL, keeping the proportion of acetonitrile in this solution to NMT 30%.
Sample solution: Dissolve 25 mg of Budesonide in 15 mL of acetonitrile in a 50-mL volumetric flask, and dilute with *Buffer* to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5500 theoretical plates, determined from the budesonide epimer B peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Budesonide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area for each impurity
r_T = sum of the areas of all of the peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
16α-Hydroxyprednisolone[a]	0.11	0.2
D-Homobudesonide[b]	0.36	0.10
21-Dehydrobudesonide (epimers)[c]	0.61; 0.66	0.07[d]
14,15-Dehydrobudesonide[e]	0.86	0.10
Total specified impurities	—	0.4[f]
Any other individual impurity	—	0.10
Total unspecified impurities	—	0.4

[a] 11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione.
[b] 16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β-hydroxy-17-(hydroxymethyl)-D-homoandrosta-1,4-diene-3,17a-dione.
[c] 16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β-hydroxy-3,20-dioxopregna-1,4-dien-21-al.
[d] Limit includes both epimers.
[e] 16α,17-[(1*RS*)-Butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4,14-triene-3,20-dione.
[f] Total specified impurities includes 11-ketobudesonide obtained in the test for *Limit of 11-Ketobudenoside* and the impurities listed above.

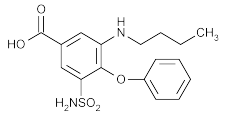
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined molds and yeast count is NMT 10^2 cfu/g.
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105 ° to constant weight: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Budesonide RS

Bumetanide



$C_{17}H_{20}N_2O_5S$ 364.42
Benzoic acid, 3-(aminosulfonyl)-5-(butylamino)-4-phenoxy-.
3-(Butylamino)-4-phenoxy-5-sulfamoylbenzoic acid [28395-03-1].

» Bumetanide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{20}N_2O_5S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25 °, excursions permitted between 15 ° and 30°.

USP Reference standards ⟨11⟩**—**
USP Bumetanide RS
USP Bumetanide Related Compound A RS
3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.
$C_{13}H_{12}N_2O_5S$ 308.31
USP Bumetanide Related Compound B RS
3-Nitro-4-phenoxy-5-sulfamoylbenzoic acid.
$C_{13}H_{10}N_2O_7S$ 338.29

USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS
$C_{21}H_{28}N_2O_5S$ 420.53

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 50 µg per mL.

Medium: isopropyl alcohol.

C: The principal spot obtained from the chromatogram of the *Test preparation* exhibits an R_F value corresponding to that in the chromatogram of *Standard solution 1,* as obtained in the test for *Related compounds.*

Loss on drying ⟨731⟩**—**Dry it at 105 ° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%, a 1-g specimen being used.

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Related compounds—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Dissolve an accurately weighed quantity of Bumetanide in methanol to obtain a solution having a concentration of about 25 mg per mL.

Standard solution 1—Dissolve an accurately weighed quantity of USP Bumetanide RS in methanol to obtain a solution having a known concentration of about 25 mg per mL.

Standard solution 2—Dilute a volume of *Standard solution 1* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

Standard solution 3—Dissolve an accurately weighed quantity of USP Bumetanide Related Compound B RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

Standard solution 4—Dissolve an accurately weighed quantity of USP Bumetanide Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.025 mg per mL.

Standard solution 5—Dissolve an accurately weighed quantity of USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.025 mg per mL.

Application volume: 20 µL of each solution.

Developing solvent system: a mixture of chloroform, cyclohexane, glacial acetic acid, and methanol (80:10:10:2.5).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. After drying the application spots, place the plate in an unlined and unsaturated chromatographic chamber. Examine the plate under short-wavelength UV light. Any secondary spots obtained from the chromatogram of the *Test solution* having R_F values corresponding to the R_F values of the principal spots obtained from the chromatograms of *Standard solutions 3, 4,* and *5* are not larger or more intense than the principal spots obtained from the chromatograms of *Standard solutions 3, 4,* and *5,* respectively: not more than 0.2% of bumetanide related compound B is found; not more than 0.1% of bumetanide related compound A is found; and not more than 0.1% of butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate is found. No other individual secondary spots obtained from the chromatogram of the *Test solution* are larger or more intense than the principal spot obtained from the chromatogram of *Standard solution 2:* not more than 0.2% of any other individual impurity is found; and not more than 0.4% of the sum of all other impurities is found (excluding bumetanide related compound A, bumetanide related compound B, and butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate).

Assay—Dissolve about 1 g of Bumetanide, accurately weighed, in 150 mL of alcohol in a 250-mL conical flask. Add phenol red TS, and titrate with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Titrimetry* ⟨541⟩), and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 36.44 mg of $C_{17}H_{20}N_2O_5S$.

Bumetanide Injection

» Bumetanide Injection is a sterile solution of Bumetanide in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bumetanide ($C_{17}H_{20}N_2O_5S$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩**—**
USP Bumetanide RS
USP Bumetanide Related Compound A RS
3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.
$C_{13}H_{12}N_2O_5S$ 308.31
USP Endotoxin RS

Identification—

A: The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay*.

B: The principal spot obtained from the chromatogram of the *Test solution* exhibits an R_F value corresponding to that of the *Identification solution,* as obtained in the test for *Related compounds*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 350 USP Endotoxin Units per mg of bumetanide.

pH ⟨791⟩: between 6.8 and 7.8.

Related compounds—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Pipet a volume of Injection, equivalent to 5 mg of bumetanide, into a 125-mL separator, and adjust with 0.1 N sodium hydroxide to a pH of 12. Extract with two 20-mL portions of ethyl ether, discard the ethyl ether extracts, and adjust the aqueous layer with 1 N acetic acid to a pH of 4. Extract with two 20-mL portions of ethyl ether, passing the extracts through anhydrous sodium sulfate. Wash the sodium sulfate with about 5 mL of ethyl ether. Evaporate the combined ethyl ether extracts with the aid of a stream of nitrogen to dryness, and dissolve the residue in 0.5 mL of methanol.

Identification solution—Dissolve USP Bumetanide RS in methanol to obtain a solution having a concentration of about 10 mg per mL.

Standard solutions—Dilute a volume of the *Identification solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.08 mg of USP Bumetanide RS per mL. Quantitatively dilute with methanol to obtain *Standard solutions* having the following compositions.

Standard solution	Dilution	Concentration (µg of RS per mL)	Percentage (%, for comparison with test specimen)
1	undiluted	80	0.8
2	3 in 4	60	0.6
3	1 in 2	40	0.4