Table 2

Related Compound	*F*	Relative Retention Time vs. Moxifloxacin	Limit (%)
Specified unknown impurity #1	1.0	0.3	0.2
Decarboxy[1]	0.13	0.4	0.3
Specified unknown impurity #2	1.0	0.9	0.3
Any specified and identified impurity	1.0	—	1.0
Other single impurities	1.0	—	0.1
*Moxifloxacin related compound A	—	1.1	—
**8-Hydroxy[2]	—	1.8	—

[1] 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-pyrrolo[3,4-b]pyridin-6-yl]-1*H*-quinolin-4-one.
[2] 1-Cyclopropyl-6-fluoro-8-hydroxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
* Disregard this peak because this is a process impurity controlled for the drug substance.
** Disregard this peak because it is quantitated using *Test 2*.

Table 3

Related Compound	*F*	Relative Retention Time vs. Moxifloxacin	Limit (%)
8-Hydroxy	0.29	1.8	0.2
Specified unknown impurity #3	1.0	3.4	0.2
Specified impurity #4*	0.42	3.9	0.2
Other single impurities	1.0	—	0.1
Total impurities (sum from both *Test 1* and *Test 2*)	—	—	1.5

*7-Amino-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.

Assay—

Buffer solution—Dissolve 0.5 g of tetrabutylammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate in 1000 mL of water, add 2 mL of phosphoric acid, filter, and degas.

Mobile phase—Use variable mixtures of *Buffer solution* and methanol as directed in *Table 1*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩.

Resolution solution—Dissolve suitable quantities of USP Moxifloxacin Hydrochloride RS and USP Moxifloxacin Related Compound A RS in *Buffer solution* to obtain a solution containing about 0.1 mg per mg and 0.001 mg per mg, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Moxifloxacin Hydrochloride RS in water to obtain a solution containing about 6 mg per mL. Dilute quantitatively, and stepwise if necessary, with the *Buffer solution* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 5 mg of moxifloxacin, to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

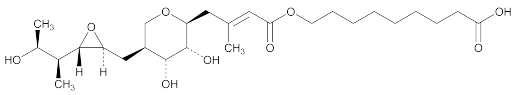
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 293-nm detector and 4.0-mm × 25-cm column that contains 5-µm packing L11. The chromatograph is programmed as shown in *Table 1*. The column temperature is maintained at 45°. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between moxifloxacin and moxifloxacin related compound A is not less than 2.0. Chromatograph the *Standard preparation,* and record peak responses as directed for *Procedure:* the column efficiency is not less than 4000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the percent of labeled amount of $C_{21}H_{24}FN_3O_4$ in the portion of the Ophthalmic Solution taken by the formula:

$$(401.43/437.89)(C_S/C_U)/(r_U/r_S)(100)$$

in which 401.43 and 437.89 are the molecular weights of moxifloxacin free base and moxifloxacin hydrochoride, respectively; C_S is the concentration, in mg per mL, of USP Moxifloxacin Hydrochloride RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of *Assay preparation* based on the label claim; r_U and r_S are the peak responses obtained from the *Assay preparation* and *Standard preparation,* respectively; and 100 is the percent factor.

Mupirocin



$C_{26}H_{44}O_9$ 500.62

Nonanoic acid, 9-[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy]-,[2*S*-2α(*E*),3β,4β,5α[2*R**, 3*R**(1*R**,[2*R**)]]]-.

(*E*)-(2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-Epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid [12650-69-0].

» Mupirocin contains not less than 920 µg and not more than 1020 µg of mupirocin ($C_{26}H_{44}O_9$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Mupirocin RS
USP Mupirocin Lithium RS

Identification—The IR absorption spectrum of a mineral oil dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mupirocin RS.

Crystallinity ⟨695⟩: meets the requirements.

pH ⟨791⟩: between 3.5 and 4.5, in a saturated aqueous solution.

Water, *Method I* ⟨921⟩: not more than 1.0%.

Assay—

pH 6.3 phosphate buffer—Prepare 0.05 M monobasic sodium phosphate, and adjust with 10 N sodium hydroxide to a pH of 6.3 ± 0.2.

Mobile phase—Prepare a suitable mixture of *pH 6.3 phosphate buffer* and acetonitrile (750:250), pass through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer about 11 mg of USP Mupirocin Lithium RS, accurately weighed, to a 100-mL volumetric flask, add 25 mL of acetonitrile, and swirl to dissolve. Dilute with *pH 6.3 phosphate buffer* to volume, and mix.

Resolution solution—Adjust 10 mL of *Standard preparation* with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 2 hours, and adjust with 5 N sodium hydroxide to a pH of 6.3 ± 0.2.

Assay preparation—Transfer about 11 mg of Mupirocin, accurately weighed, to a 100-mL volumetric flask, add 25 mL of acetonitrile, and swirl to dissolve. Dilute with *pH 6.3 phosphate buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 25-cm column that contains packing L1 based on spherical silica particles. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for the mupirocin acid hydrolysis product and 1.0 for mupirocin, and the resolution, *R,* between the mupirocin acid hydrolysis product and mupirocin 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, the column efficiency is not less than 1500 theoretical plates when calculated by the formula:

$$5.545(t_r / W_{h/2})^2$$

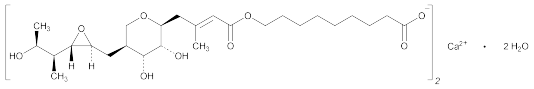
in which the terms are as defined therein. The relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] 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 µg, of mupirocin ($C_{26}H_{44}O_9$) in each mg of Mupirocin taken by the formula:

$$(M_S E / M_U)(r_U / r_S)$$

in which M_S is the weight, in mg, of USP Mupirocin Lithium RS taken to prepare the *Standard preparation; E* is the mupirocin equivalent, in µg per mg, of USP Mupirocin Lithium RS; M_U is the weight, in mg, of mupirocin taken to prepare the *Assay preparation;* and r_U and r_S are the mupirocin peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Mupirocin Calcium



$C_{52}H_{86}CaO_{18} \cdot 2H_2O$ 1075.34

Nonanoic acid, 9-[[3-Methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy-, calcium salt (2:1), dihydrate, [2*S*-[2α(*E*),3β,4β,5α[2*R**,3*R**(1*R**,2*R**)]]]-.

(α*E*,2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-Epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid, calcium salt (2:1), dihydrate [115074-43-6].

» Mupirocin Calcium contains the equivalent of not less than 865 µg and not more than 936 µg of mupirocin ($C_{26}H_{44}O_9$) per mg.

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

USP Reference standards ⟨11⟩**—**
USP Mupirocin Calcium RS
USP Mupirocin Lithium RS

Identification—

A: *Infrared Absorption* ⟨197M⟩—[NOTE—Do not dry or grind extensively.]

B: 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.*

C: When moistened with hydrochloric acid, it meets the requirements of the flame test for *Calcium* ⟨191⟩.

Specific rotation ⟨781S⟩**:** between –16° and –20°.

Test solution: 50 mg per mL, in methanol.

Water, *Method I* ⟨921⟩**:** not less than 3.0% and not more than 4.5%.

Chloride ⟨221⟩**—**Dissolve 50 mg in a mixture of 1 mL of 2 N nitric acid and 15 mL of methanol. Add 1 mL of silver nitrate TS: the turbidity does not exceed that produced by 0.70 mL of 0.020 N hydrochloric acid (0.5%).

Related compounds—

0.1 M Ammonium acetate—Prepare as directed in the *Assay.*

Mobile phase—Prepare a filtered and degassed mixture of *0.1 M Ammonium acetate* and tetrahydrofuran (70:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

pH 4 Acetate buffer—Transfer about 13.6 g of sodium acetate to a 1000-mL volumetric flask, and dissolve in about 900 mL of water. Adjust with glacial acetic acid to a pH of 4.0, and dilute with water to volume.

Diluent—Prepare a mixture of *pH 4 Acetate buffer* and methanol (1:1).

Standard solution—Transfer about 25 mg of USP Mupirocin Lithium RS, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Test solution—Transfer about 50 mg of Mupirocin Calcium, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Resolution solution—Adjust 10 mL of the *Standard solution* with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 20 hours, and adjust with 5 N sodium hydroxide to a pH of 4.0.

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 L7. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* two peaks are observed at retention times of about 0.63