Table 2

Name	Relative Retention Time	Relative Response Factor	Wavelength (nm)	Acceptance Criteria, NMT (%)
Moxifloxacin related compound F	0.82	1.0	293	—[a]
Moxifloxacin	1.0	—	293/317	—
Moxifloxacin related compound A	1.1	0.53	293	—[a]
Moxifloxacin related compound B[b]	1.26	0.77	317	—[a]
Moxifloxacin related compound C[c]	1.33	1.0	293	—[a]
Moxifloxacin related compound D[d]	1.38	0.76	293	—[a]
Moxifloxacin related compound E[e]	1.49	0.26	293	—[a]
8-Hydroxy quinolonic acid derivative[f]	1.72	1.3	293	—[a]
8-Methoxy quinolonic acid derivative[g]	1.89	1.9	317	—[a]
8-Methoxy quinolonic ethyl ester[h]	1.93	1.6	317	—[a]
Any other individual impurity	—	1.0	293	0.2
Total impurities	—	—	293/317	0.75

[a] For identification only. These are process related impurities monitored in the drug substance and not included in the total impurities calculation.

[b] 1-Cyclopropyl-6,8-dimethoxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.

[c] 1-Cyclopropyl-8-ethoxy-6-fluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.

[d] 1-Cyclopropyl-8-fluoro-6-methoxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.

[e] 1-Cyclopropyl-6-fluoro-8-hydroxy-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.

[f] 1-Cyclopropyl-6,7-difluoro-8-hydroxy-4-oxo-3-quinolinecarboxylic acid.

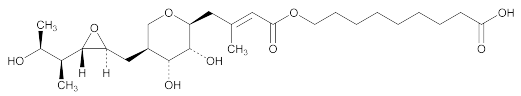
[g] 1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-3-quinolinecarboxylic acid.

[h] Ethyl 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-3-quinolinecarboxylate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature. Avoid high humidity.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Moxifloxacin Hydrochloride RS
  USP Moxifloxacin Related Compound A RS
  1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.
  $C_{20}H_{21}F_2N_3O_3$ 389.40
  USP Moxifloxacin Related Compound F RS
  1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-1-methylhexahydro-1*H*-pyrrolo[3,4-*b*]pyridin-6(2*H*)-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
  $C_{22}H_{26}FN_3O_4$ 415.46

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 Determination, *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:

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

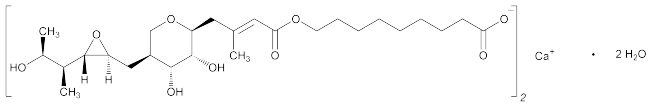
in which the terms are as defined therein. The relative standard deviation for replicate injections is not more than 2.0%.

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].

DEFINITION

Mupirocin Calcium contains the equivalent of NLT 865 µg/mg and NMT 936 µg/mg of mupirocin ($C_{26}H_{44}O_9$).

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197M⟩**
  Sample: Do not grind extensively.
  Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL ⟨191⟩, *Calcium*:** Meets the requirements

ASSAY

- **PROCEDURE**
  Solution A: 7.7 g/L of ammonium acetate in water, adjusted with glacial acetic acid to a pH of 5.7 before diluting to the final volume
  Mobile phase: Tetrahydrofuran and *Solution A* (32:68)
  Standard solution: 125 µg/mL of USP Mupirocin Lithium RS prepared as follows. Transfer a suitable amount of USP Mupirocin Lithium RS to a suitable volumetric flask, dissolve in methanol, using 2.5% of the final volume, and dilute with *Solution A* to volume.
  System suitability solution: Adjust 10 mL of the *Standard solution* with 6 N hydrochloric acid to a pH of 2.0, and allow to stand for 20 h.
  Sample solution: Transfer 25 mg of Mupirocin Calcium to a 200-mL volumetric flask, dissolve in 5 mL of methanol, and dilute with *Solution A* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 230 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 7.0 between the second of the two peaks corresponding to mupirocin rearrangement products and the peak corresponding to mupirocin, *System suitability solution*
  Relative standard deviation: NMT 1.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the quantity, in µg/mg, of mupirocin ($C_{26}H_{44}O_9$) in the portion of Mupirocin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area of mupirocin from the *Sample solution*
r_S = peak area of mupirocin from the *Standard solution*
C_S = concentration of USP Mupirocin Lithium RS in the *Standard solution* (mg/mL)
C_U = concentration of Mupirocin Calcium in the *Sample solution* (mg/mL)
P = potency of mupirocin in USP Mupirocin Lithium RS (µg/mg)

Acceptance criteria: 865–936 µg/mg

IMPURITIES

- **CHLORIDE AND SULFATE ⟨221⟩, *Chloride***
  Analysis: 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.
  Acceptance criteria: The turbidity does not exceed that produced by 0.70 mL of 0.020 N hydrochloric acid (0.5%).
- **ORGANIC IMPURITIES**
  Solution A: Prepare as directed in the *Assay*.
  Solution B: 13.6 g/L of sodium acetate in water, adjusted with glacial acetic acid to a pH of 4.0 before diluting to the final volume
  Mobile phase: Tetrahydrofuran and *Solution A* (30:70)
  Diluent: Methanol and *Solution B* (1:1)
  Standard solution: 125 µg/mL of USP Mupirocin Lithium RS in *Diluent*
  System suitability solution: Adjust 10 mL of the *Standard solution* with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 20 h, and adjust with 5 N sodium hydroxide to a pH of 4.0.
  Sample solution: 5 mg/mL of Mupirocin Calcium in *Diluent*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 240 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Flow rate: 1 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  [NOTE—The relative retention times for two mupirocin rearrangement products and mupirocin in the *System*