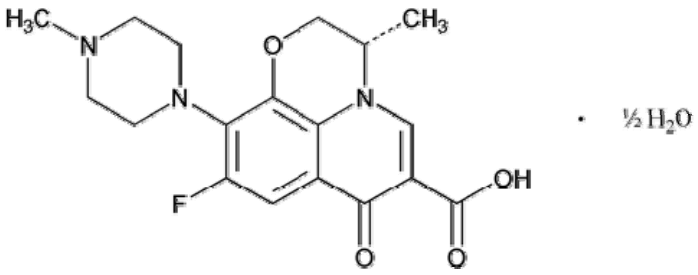
Levofloxacin

USP Method Levofloxacin RS
USP Method Levofloxacin Assay



Manufacturer: Sanofi-Aventis
Johnson and Johnson /Ortho-McNeil (US)
(license from Daiichi Sankyo Co., Ltd - Patent expired 2010)

Brand Name: Tavanic, Levaquin Oftaquix, Quixin, Iquix, Levores

Levofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe or life-threatening bacterial infections or bacterial infections that have failed to respond to other antibiotic classes.

Levofloxacin is used to treat a number of infections including: respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, endocarditis, meningitis, pelvic inflammatory disease, and traveler's diarrhea

Levofloxacin is considered to be same as Ofloxacin by the U.S. Food and Drug Administration (FDA), with the exception of the potency shown in vitro against mycobacteria.

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Levofloxacin

USP34 – NF29 S1

USP Columns:

Inertsil ODS-3 Assay and Organic Impurities 4.6 mm x 25 cm, 5 µm.
An alternative column is Prodigy ODS(3) in the same dimensions, Phenomenex

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm (1.51456.0001)

Recommended Solvents and Reagents:

Methanol for liquid chromatography LiChrosolv® (1.06018)

Water Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q water purification system

L-isoleucine (1.05362)
Ammonium Acetate Use ACS Reagent grade
Cupric Sulfate, pentahydrate Use ACS Reagent grade

USP Standards

Levofloxacin (200 mg)	USP Product Number:	1362103
Levofloxacin Related Compound A (25 mg)	USP Product Number:	1362114
Levofloxacin Related Compound B (35 mg)	USP Product Number:	1362125

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USP Method for Levofloxacin Assay

Solution A
8.5 g/L of ammonium acetate, 1.25 g/L of cupric sulfate, pentahydrate and 1.3 g/L of l-isoleucine in water

Mobile phase
Methanol and Solution A (3:7)

Standard solution
1 mg/mL of USP Levofloxacin RS in Mobile phase

Sample solution
1 mg/mL of Levofloxacin in Mobile phase

Chromatographic system (*See Chromatography 621, System Suitability*)
Detector: UV 360 nm
Column temperature: 45°C
Injection size: 25 µL
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 0.8 mL/min

System suitability
Sample: Standard solution

Suitability requirements
Tailing factor: 0.5–1.5
Relative standard deviation: Not more then (NMT) 1.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of $C_{18}H_{20}FN_3O_4$ in the portion of Levofloxacin taken:

$$\text{Result} = (rU/rS) \times (CS/CU) \times 100$$

rU = peak response of Levofloxacin from the Sample solution
rS = peak response of levofloxacin from the Standard solution
CS= concentration of USP Levofloxacin RS in the Standard solution (mg/mL)
CU= concentration of Levofloxacin in the Sample solution (mg/mL)

Acceptance criteria
98.5%–102.0% on the anhydrous basis

USP Method for Levofloxacin RS

Solution A, Mobile phase, Sample solution, and Chromatographic system

Proceed as directed in the Assay.

System suitability solution: 1 mg/mL of USP Levofloxacin RS in Mobile phase
Sensitivity solution: 0.3 µg/mL of USP Levofloxacin RS in Mobile phase

System suitability *(Samples: System suitability solution and Sensitivity solution)*

Suitability requirements
Relative standard deviation: NMT 1.0%, System suitability solution
Signal-to-noise ratio: not less then (NLT) 10, Sensitivity solution

Analysis (*Sample: Sample solution*)

Calculate the percentage of each individual impurity in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak area response of each impurity
r_S = peak area response of levofloxacin
F = relative response factor (see Impurity Table 1)

Acceptance criteria (*Individual impurities: See Impurity Table 1*)

Total impurities: NMT 0.5%. [Note—Do not include the d-isomer in the calculation for Total impurities.]

Table 1.

Name	RRT	Relative Response Factor	Acceptance Criteria NMT (%)
N-Desmethyl levofloxacin[a]	0.47	1.0	0.3
Diamine derivative[b]	0.52	0.9	0.3
Levofloxacin N-oxide[c]	0.63	1.1	0.3
9-Desfluoro levofloxacin[d]	0.73	1.0	0.3
Levofloxacin	1.0	—	—
d-Isomer[e]	1.23	1.0	0.8
Any unknown impurity	—	1.0	0.1

a: (S)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

b: (S)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid

c:(S)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido-[1,2,3-de][1,4]benzoxazine-10-yl)-1-methyl-piperazine-1-oxide.

d: (S)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

e: (R)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

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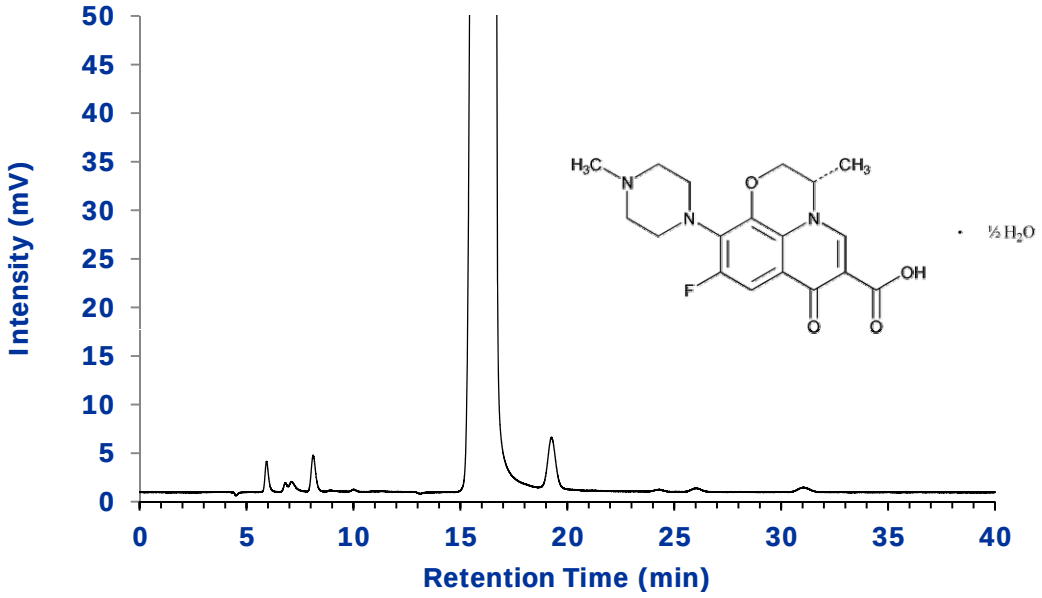
USP Method for Levofloxacin

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	25 µL	
Detection:	Shimadzu Prominence, UV 360 nm	
Cell:	10 µL	
Flow Rate:	0.8 mL/min	
Mobile Phase (v/v):	Buffer: 8.5 g/L of ammonium acetate, 1.25 g/L of cupric sulfate, pentahydrate and 1.3 g/L of L-isoleucine in water. Mix buffer and methanol 70:30.	
Temperature:	45°C	
Diluent	Mobile phase	
Sample:	System suitability solution: 1 mg/mL of Levofloxacin	
Pressure Drop:	112 Bar (1624 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Asymmetry (T_{USP})
1	N-Desmethyl Levofloxacin	5.9	0.4	1.4
2	Diamine derivative	8.1	0.5	1.3
3	**Levofloxacin**	**16.5**	**1.0**	**0.6**
4	D-isomer	19.3	1.2	1.1

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