United States Pharmacopeia Methods
Purospher® STAR Columns for Regulated Drug Analysis

www.merck-chemicals.com/chromatography

Merck Millipore is a division of MERCK

Introduction

The process of validating a new analytical procedure for compendial usage is addressed in United States Pharmacopea (USP) general Chapter <1225> "Validation of Compendial Procedures". However, even with a fully validated procedure, the scientists may not have assurance that the procedure is suitable for use with a specific ingredient or product in a specific laboratory with specific personnel, equipments, consumables and reagents. USP therefore developed chapter <1226> in response to industry's request to provide instructions for verifying compendial procedures in specific situations.

In this compilation, we have addressed USP's proposed new general chapter <1226> "Verification of Compendial Procedures" which is intended to fill the gap in the proper usage of compendial procedures by outlining a process for verifying their suitability.

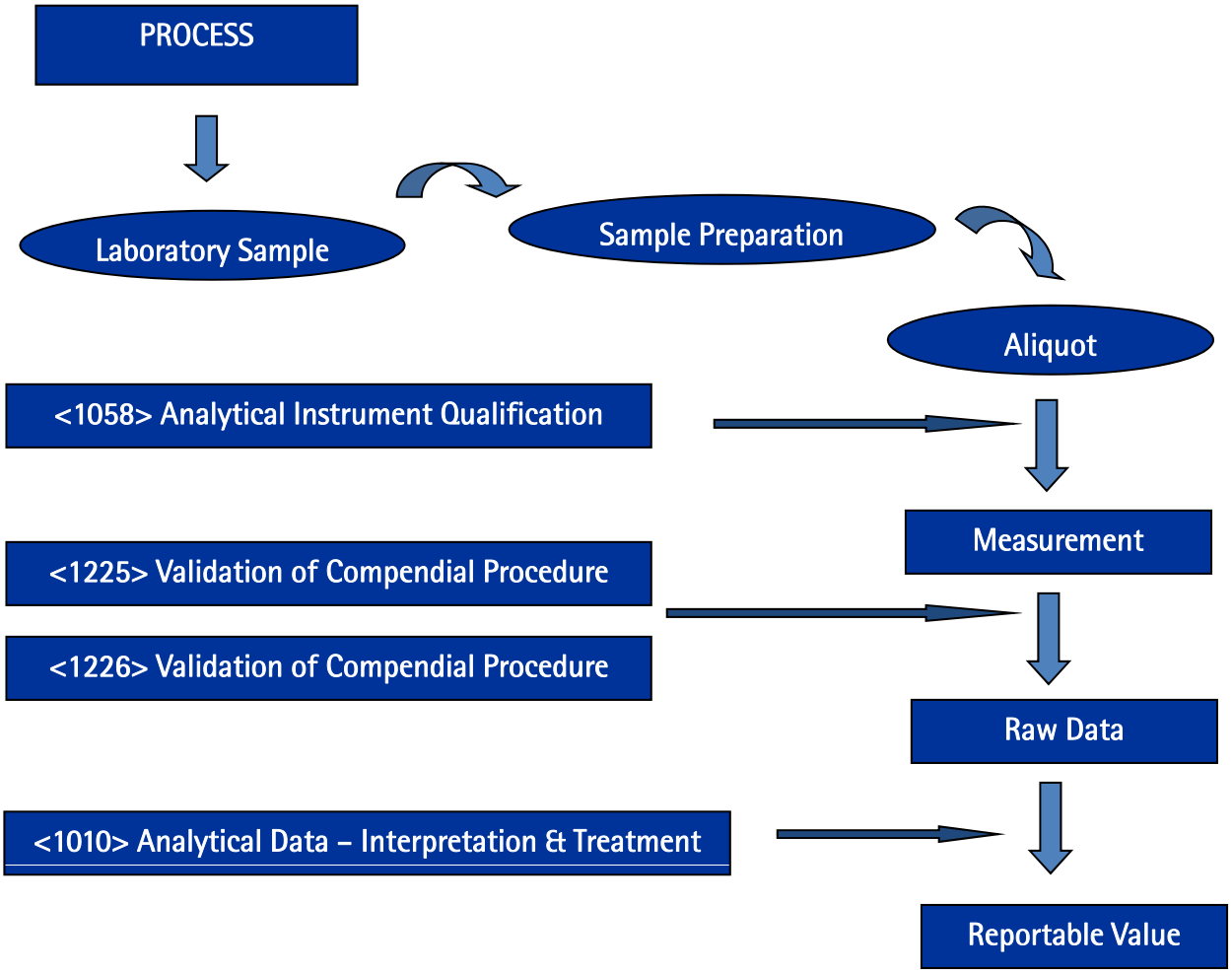
The role of HPLC columns is of immense importance to meet system suitability test (SST) criteria in compendial methods. This is illustrated in this complation by analysing important drugs on Purospher® STAR as per pending USP monographs.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Content

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

The Analytical Process



Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Validation of Compendial Procedure <1225>

1. Defines analytical performance characteristics
2. Recommends data for submission to USP-NF
3. Provides guidance on which analytical performance characteristics are needed based on the type of test
4. Incorporates ICH guidelines Q2A and Q2B

Performance Characteristics	Category I	Category II		Category III	Category IV
		Quant	Limit Test		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
LOD	No	No	Yes	*	No
LOQ	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

Verification of Compendial Procedures <1226>

The intention of this USP chapter is to provide general information to laboratories on the verification of compendial procedures that are being performed for the first time to yield acceptable results utilizing the laboratories' personnel, equipment, and reagents.

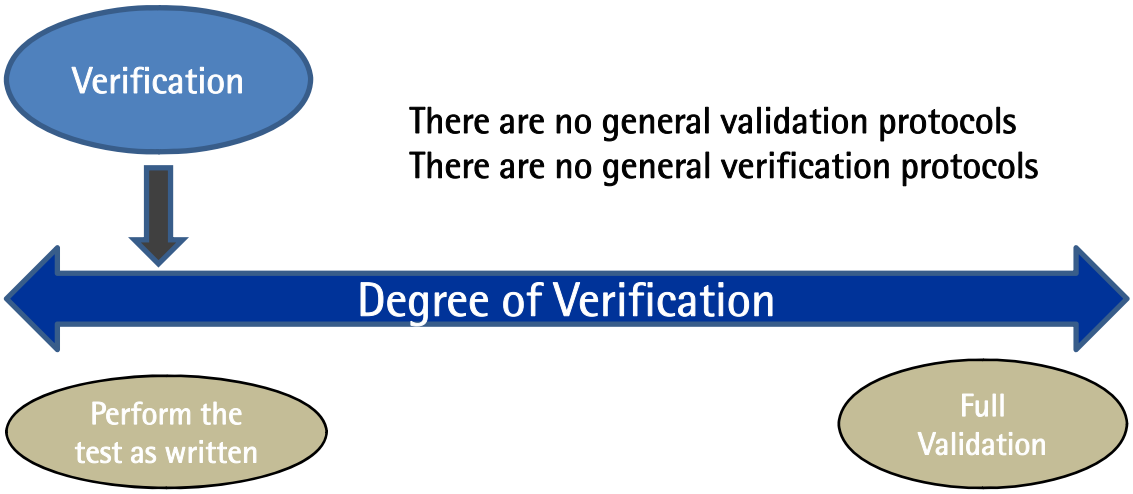
This is not applicable for retroactive application to already successfully established laboratory procedures. Verification consists of assessing selected Analytical Performance Characteristics, such as those which are described in chapter <1225>, to generate appropriate, relevant data rather than repeating the validation process.

Why USP <1226> is needed

1. "21 CFR211.194 (a)(2): "users of analytical methods described in USP–NF are not required to validate the accuracy and reliability of these methods, but merely verify their suitability under actual conditions of use.

2. Response to industry inquiries

3. Verification consist of assessing selected Analytical Performance Characteristics, such as those which are described in USP Chapter <1225>, to generate appropriate, relevant data rather than repeating the validation process.



Case Study: Related Substance in API

Identification
- IR,UV
- HPLC

Purity Test
- Loss on drying, residue on ignition
- Heavy metals
- pH
- Related substances (RS)

Assay
- Titrimetry
- HPLC

Performance	Validation	Verification
Accuracy	Yes	No
Precision	Yes	Maybe
Specificity	Yes	Yes
LOD	No	No
LOQ	Yes	Yes
Linearity	Yes	No
Range	Yes	No

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

System Suitability Test (SST)

1. SST is used to verify that the chromatographic system is adequate for the intended analysis
2. SST is based on the concept that the equipment, electronics, analytical operations, and samples analyzed constitute an integral system that can be evaluated as such

Factors that may affect chromatographic behavior

• Composition, ionic strength, temperature, and apparent pH of the mobile phase
• Flow rate, column dimensions, column temperature, and pressure
• Stationary phase characteristics, including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, and specific surface area
• Reversed-phase and other surface modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading, etc.)

Aqueous Buffer pH

The pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within ±0.2 units of the value or range specified.

Concentration of Salts in Buffer

The concentration of the salts used in the preparation of the aqueous buffer employed in the mobile phase can be adjusted to within ±10% if the permitted pH variation (see above) is met.

Ratio of Components in Mobile Phase

The following adjustment limits apply to minor components of the mobile phase (specified at 50% or less). The amounts of these components can be adjusted by ±30% relative. However, the change in any component cannot exceed ±10% absolute (i.e., in relation to the total mobile phase). Adjustment can be made to one minor component in a ternary mixture. Examples of adjustments for binary and ternary mixtures are given below.

Binary Mixtures specified ratio of 50:50: 30% of 50 is 15% absolute, but this exceeds the maximum permitted change of ±10% absolute in either component. Therefore, the mobile phase ratio may be adjusted only within the range of 40:60 to 60:40.
• specified ratio of 2:98: 30% of 2 is 0.6% absolute. Therefore the maximum allowed adjustment is within the range of 1.4:98.6 to 2.6:97.4.

Ternary Mixtures specified ratio of 60:35:5: For the second component, 30% of 35 is 10.5% absolute, which exceeds the maximum permitted change of ±10% absolute in any component. Therefore the second component may be adjusted only within the range of 25% to 45% absolute. For the third component, 30% of 5 is 1.5% absolute. In all cases, a sufficient quantity of the first component is used to give a total of 100%. Therefore, mixture ranges of 50:45:5 to 70:25:5 or 58.5:35:6.5 to 61.5:35:3.5 would meet the requirement.

Wavelength of UV-Visible Detector

Deviations from the wavelengths specified in the procedure are not permitted. The procedure specified by the detector manufacturer, or another validated procedure, is used to verify that error in the detector wavelength is, at most, ±3 nm.

Stationary Phase

Column length

Can be adjusted by as much as ±70%.

Column Inner diameter

Can be adjusted if the linear velocity is kept constant. See Flow Rate below.

Particle Size

The particle size can be reduced by as much as 50%, but cannot be increased.

Flow Rate

When column dimensions have been modified, the flow rate can be adjusted using:

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2}$$

F_1 is the flow rate indicated in the monograph, in mL/min;
F_2 is the adjusted flow rate, in mL/min;
l_1 is the length of the column indicated in the monograph;
l_2 is the length of the column used;
d_1 is the column inner diameter indicated in the monograph
d_2 is the internal diameter of the column used.

Additionally, the flow rate can be adjusted by ±50%.

Injection Volume

The injection volume can be reduced as far as is consistent with accepted precision and detection limits. No increase is permitted.

Column Temperature

The column temperature can be adjusted by as much as ±10 °C.
Column thermostating is recommended to improve control and reproducibility of retention time.

HPLC Column Selection

USP has created a database for classification of chromatography columns to help users of chromatography to cross reference HPLC columns that can be equivalent to the one in current use.

In this context Merck Millipore offers the following range of HPLC columns in C18/C8 chemistries to fullfil SST criteria in the compendial methods :

1. Purospher® STAR
2. Chromolith®
3. Lichrospher®
4. Superspher ®

General tests like water content, heavy metals, residue on ignition do not typically require verfication.

Summary of Compilation

In this compliation several "block buster" molecules have been tested with their corresponding Assay and Related Substances (RS) USP method.

The results herein illustrate the excellence in performance of Purospher® STAR columns. Combined with the outstanding quality (batch-to-batch reproducibility) of masterbatch production, the data proves that Purospher® STAR columns are indeed useful for regulated pharmaceutical analysis.

A majority of the molecules have been tested in either the Assay or RS method, and others have been analysed according to the complete monograph.

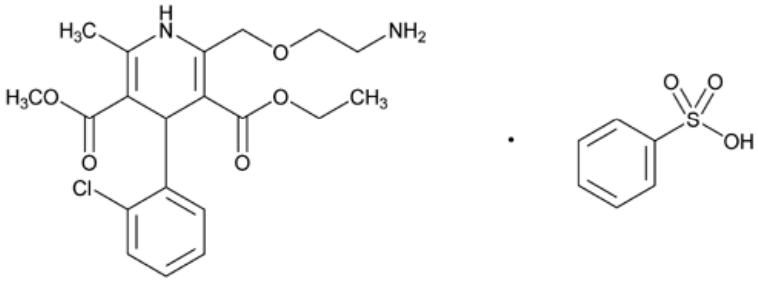
Some of the molecules have complete analysis protocols to verify system suitability, including determination of limit of detection (LOD), limit of quantitation (LOQ), method linearity, etc.

A few molecules have been tested with the given monograph, and then scaled to a narrower inner diameter with adjusted flow rate.

There are also examples of molecules tested with the given monograph, and then scaled to a column with narrower inner diameter with adjusted flow rate and with reduced particle size.

Amlodipine

USP Method Amlodipine Besylate Assay
USP Method Amlodipine Besylate RS



Original Manufacturer	Pfizer (Patent expired 2007)
Original Brand Name	Norvasc
Generic Names	Aforbes, Agen, Aken, Amlosun, Amcard, Amdepin, Amdipin, Amlodine, Amlodipine 5, Amlopin, Amlopine, Amlovasc, Asomex, ATECARD-AM, Camlodin, Dailyvasc, Istin, Lodopin, Lopin, Nopidin, Perivasc, Tenox...
Combination Drugs	Exforge (Amlodipine and Valsartan) Lotrel (Amlodipine and Benazepril) Caduet (Amlodipine and Atorvastatin)

Amlodipine (as besylate, mesylate or maleate) is a calcium channel blocker, and used as an anti-hypertensive and for treatment of angina. Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance, thereby reducing blood pressure.

In angina it increases blood flow to the heart muscle.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Amlodipine Besylate

USP34 – NF29 S1

USP Columns

Symmetry C18 Assay and Related Compounds 3.9 mm x 15 cm, µm, Waters

Nova-Pak C18 Assay and Related Compounds Alternative column. 3.9 mm x 15 cm, 4 µm, Waters

Nova-Pak C18 Chromatographic purity 15 cm x 3.9 mm, 4 µm, Waters

Equivalent Column

Purospher® STAR RP-18 endcapped (5 µm) 125×4.0 mm (1.50036.0001)

Recommended Solvents and Reagents

Methanol	for liquid chromatography LiChrosolv®	(1.06018)
Acetonitrile	isocratic grade for liquid chromatography LiChrosolv®	(1.14291)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	(1.15333)

Triethylamine Use a suitable grade with a content of not less than 99.5%. (8.45061)

Phosphoric Acid Use ACS reagent grade

USP Standards

Amlodipine Besylate (350 mg)	USP Product Number:	1012928
Amlodipine Related Compound A (25 mg)	USP Product Number:	1029501

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method Amlodipine Besylate Assay

Buffer
Dissolve 7.0 mL of triethylamine in 800 mL of water.
Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and dilute with water to 1 L.

Mobile phase
Prepare a filtered and degassed mixture of pH 3.0 Buffer, methanol, and acetonitrile (50:35:15). Make adjustments if necessary (*see System Suitability under Chromatography 621*).

Standard preparation
Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in Mobile phase to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay preparation
Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.
Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix.

Chromatographic system
The liquid chromatograph is equipped with a 237 nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the standard deviation for replicate injections is not more than 2.0%.

Procedure
Separately inject equal volumes (about 10 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 percentage of Amlodipine Besylate using the formula:

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

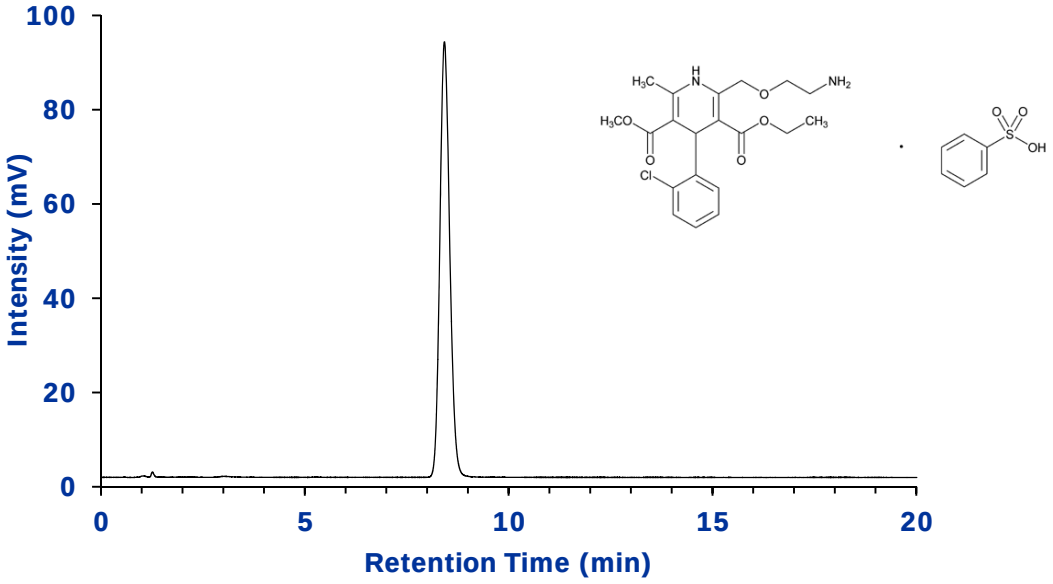
in which C_S and C_U are the concentrations, in mg per mL, of amlodipine besylate in the Standard preparation and the Assay preparation, respectively; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method for Amlodipine Besylate Assay

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 125x4.0 mm	1.50036.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence, UV 237 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 7.0 ml triethyl amine in 800 ml of water. pH adjusted to 3.0 +/- 0.1 with phoshoric acid. Dilute to 1 liter with water. Mix. Buffer, Methanol and Acetonitrile 50:35:15(v/v)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	40 ppm of Amlodipine in mobile phase	
Pressure Drop:	131 Bar (1900 psi)	



Chromatographic Data

No.	Compound	Time (min)	Theoretical Plate	Tailing Factor
1	Amlodipine Besylate	8.4	5787	1.15

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method Amlodipine Besylate RS

Buffer and mobile phase (*Prepare as directed in the Assay.*)

System suitability solution
Dissolve about 5 mg of Amlodipine Besylate in 5 mL of hydrogen peroxide, and heat at 70 degrees Celsius for 45 minutes.

Standard solution
Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in Mobile phase to obtain a solution having a known concentration of about 0.003 mg per mL.

Test solution
Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

Chromatographic system
Prepare as directed in the Assay. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the resolution, Rs, between amlodipine impurity A and amlodipine is not less than 4.5. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the standard deviation for replicate injections is not more than 10.0%.

[note—For the purpose of identification, the relative retention times are about 0.2 for benzene sulfonate, 0.5 for amlodipine impurity A, and 1.0 for amlodipine. Amlodipine impurity A is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate.]

Procedure
Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms for a period of time that is about 3 times the retention time of amlodipine, and measure the peak responses. Calculate the percentage of each impurity in the portion of Amlodipine Besylate taken by the formula:

$$100(1/F)(C_S/C_T)(r_i/r_S)$$

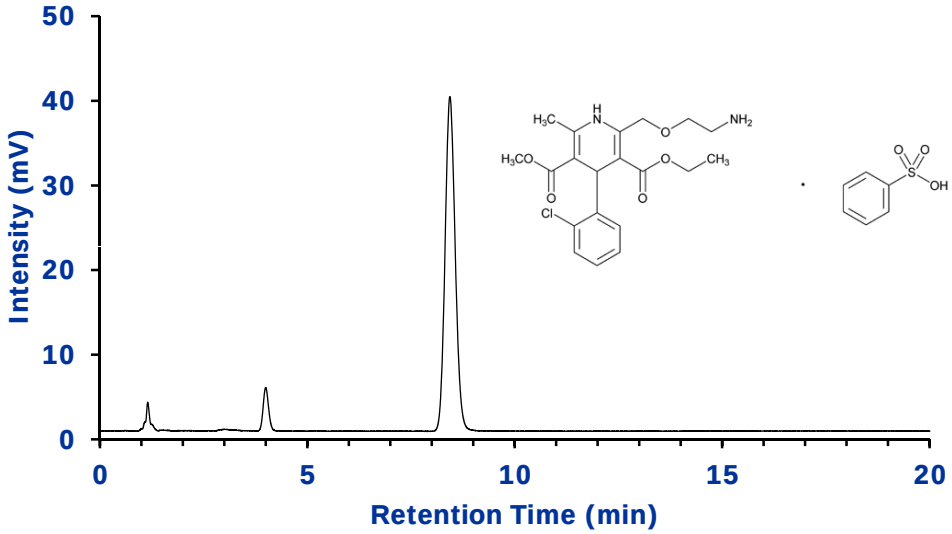
in which F is the relative response factor, which is equal to 0.5 for amlodipine impurity A and to 1.0 for other impurities; C_S and C_T are the concentrations, in mg per mL, of amlodipine besylate in the Standard solution and the Test solution, respectively; r_i is the peak response for each impurity obtained from the Test solution; and r_S is the peak response for amlodipine besylate obtained from the Standard solution: not more than 0.3% of amlodipine impurity A is found, and not more than 0.3% of total other impurities is found. Disregard any peak less than 0.03%, and disregard any peak due to benzene sulfonate.

USP Method for Amlodipine Besylate RS

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 125x4.0 mm	1.50036.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence, UV 237 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 7.0 ml triethyl amine in 800 ml of water. pH adjusted to 3.0 +/- 0.1 with phoshoric acid. Dilute to 1 liter with water. Mix. Buffer, Methanol and Acetonitrile 50:35:15(v/v)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	40 ppm of Amlodipine and 3 ppm of Amlodipine RS A in mobile phase	
Pressure Drop:	131 Bar (1900 psi)	



Chromatographic Data

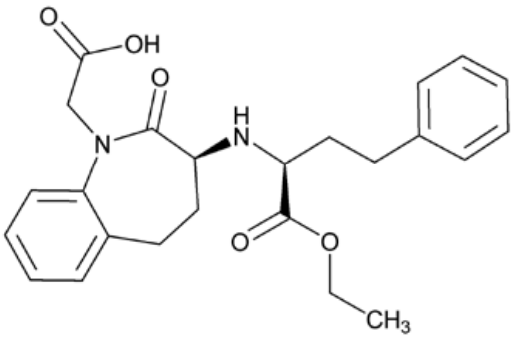
No.	Compound	Time (min)	Resolution	Relative Retention Time (RRT)
1	Amlodipine Related Compound A *	4.0	0.0	0.5
2	Amlodipine Besylate	8.4	12.9	1.0

•3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].
The relative retention times should be 0.2 for benzene sulfonate, 0.5 for amlodipine impurity A, and 1.0 for amlodipine.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Benazepril

USP Method Benazepril RS



• HCl

Original Manufacturer:	Novartis (basic Benazepril substance patent expired 2007)
Original Brand Name:	Lotensin®
Generic Producers:	Mylan Laboratories, Ranbaxy Pharmaceuticals Sandoz, Teva Pharmaceuticals
Combination Drugs:	Lotrel (Amlodipine and Benazepril)

Benazepril is used to treat high blood pressure (hypertension), congestive heart failure, and chronic renal failure

Under the brand names Fortekor (Novartis) and VetACE (Jurox Animal Health), Benazepril hydrochloride is used to treat congestive heart failure in dogs and chronic renal failure in dogs and cats.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Benazepril Hydrochloride

USP34 – NF29 S1

USP Columns - Assay and Related Compounds (RS) Test 2:

MicroBondapak C18 Analytical column 3.9 mm x 300 mm, guard column 4.6 mm x 30 mm,

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 250x4.0 mm, (1.50037.0001)

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

Tetrabutylammonium Bromide Use ACS reagent grade

USP Standards

Benazepril Hydrochloride (125 mg)	USP Product Number:	1048619
Benazepril Related Compound B (15 mg)	USP Product Number:	1048630
Benazepril Related Compound C (50 mg)	USP Product Number:	1048641
Benazepril Related Compound D (15 mg)	USP Product Number:	1048652
Benazepril Related Compound E (25 mg)	USP Product Number:	1048663
Benazepril Related Compound F (15 mg)	USP Product Number:	1048674
Benazepril Related Compound G (15 mg)	USP Product Number:	1048685

USP Method for Benazepril HCl RS

Buffer
Dissolve 0.81 gram of tetrabutylammonium bromide in 360 mL of water containing 0.2 mL of glacial acetic acid.

Mobile phase
Prepare a filtered and degassed mixture of methanol and Tetrabutylammonium bromide solution (64:36). Make adjustments if necessary (see System Suitability under Chromatography 621).

System suitability solution
Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound B RS in Mobile phase to obtain a solution having known concentrations of about 0.4 mg of each per mL.

Standard solution (*Test 2 for Benazepril related compounds B, C, D, E, F, and G)*
Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS, USP Benazepril Related Compound B, C, D, E, F, and G RS in Mobile phase to obtain a solution having known concentrations of about 1 µg of USP Benazepril Hydrochloride RS per mL and 10 µg of each related compound per mL.

Procedure
Separately inject equal volumes (about 25 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the percentage of Benazepril related compounds in the portion of Benazepril Hydrochloride taken by the formula: $100(C_S / C_T)(r_U / r_S)$

C_S = conc. in mg/mL, of the relevant USP Reference Standard in the Standard solution
C_T = conc. in mg/mL, of Benazepril hydrochloride in the Test solution
r_U = peak response for the relevant Benazepril related compound obtained from the Test solution
r_S = peak response for the relevant Benazepril related compound obtained from the Standard solution (see Table 1 for values).

Chromatographic system
The liquid chromatograph is equipped with a 240-nm detector and a 4.6×30 mm guard column that contains packing L1 connected to a 3.9×300 mm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the resolution, R, between Benazepril hydrochloride and Benazepril related compound B is not less than 1.7; and the relative standard deviation for replicate injections determined from Benazepril hydrochloride and Benazepril related compound B is not more than 2.0% for each.

USP Method for Benazepril HCl RS

Table 1.

No.	Compound	Relative Retention Time (RRT)	Limit (%)
1	Impurity E	0.4	0.2
2	Impurity F	0.5	0.2
3	Impurity C	0.6	0.3
4	Impurity B	1.5	0.5
5	Impurity D	1.7	0.2
6	Impurity G	2.0	0.2

Impurity E	3-Amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid
Impurity F	t-Butyl-3-amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid
Impurity C	3-(1-Carboxy-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid
Impurity B	Mixture of diastereoisomers (3-(1-ethoxycarbonyl-3-phenyl-(1R)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid and (3-(1-ethoxycarbonyl-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3R)-benzazepine)-1-acetic acid
Impurity D	3-(1-Ethoxycarbonyl-3-cyclohexyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid monohydrochloride
Impurity G	3-(1-Ethoxycarbonyl-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid ethyl ester

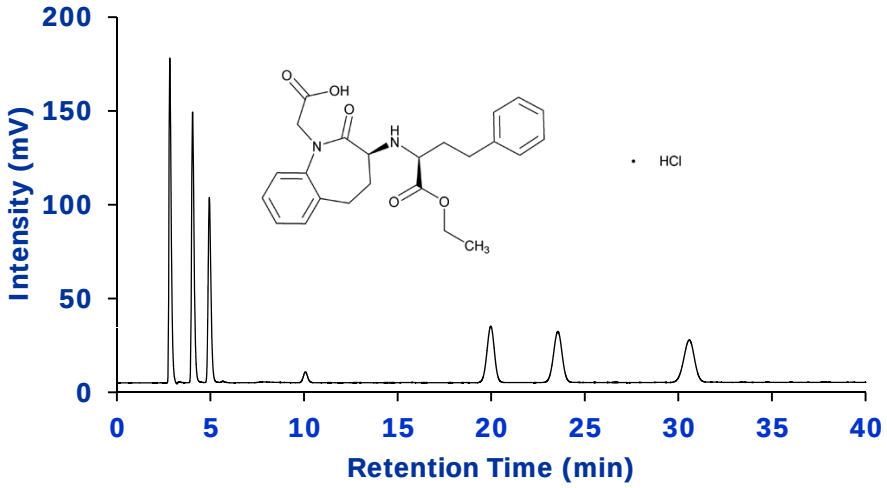
In addition to not exceeding the limits for benazepril related compounds in Table 1, not more than 0.1% of any other single impurity is found; [note—For calculating any other single unspecified impurity, C_S is the concentration of the USP Benazepril Hydrochloride RS in the Standard solution.] and not more than 2.0% of total impurities (excluding benazepril related compound A from Test 1) is found.

USP Method for Benazepril HCl RS

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x4.0 mm	1.50037.0001
Injection:	25 µL	
Detection:	Shimadzu Prominence, UV 240 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 0.81 gram of tetrabutyl ammonium bromide in 360 ml water containing 0.2 ml acetic acid. Mix. Buffer and Methanol 36:64.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	Benazepril (1 ppm) + imp B, C, D, E, F and G (10 ppm each)	
Pressure Drop:	200 Bar (2900 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Impurity E	2.8	0.3	0.0	1.6
2	Impurity F	4.0	0.4	5.3	1.4
3	Impurity C	4.9	0.5	3.5	1.3
	Benazepril	10.1	1.0	14.7	1.1
4	Impurity B	20.0	2.0	17.1	1.0
5	Impurity D	23.6	2.3	4.5	1.0
6	Impurity G	30.6	3.0	7.3	1.0

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Analysis protocol for Benazepril

USP Method Repeatability

No.	Compound	Response (Arbitrary Area Units)	Relative Standard Deviation (%)	N
1	Impurity E	1456487	0.1	5
2	Impurity F	1334201	0.6	5
3	Impurity C	1021527	0.6	5
	Benazepril	92267	1.3	5
4	Impurity B	834757	0.1	5
5	Impurity D	868154	0.3	5
6	Impurity G	893672	0.3	5

Replicate injections of standard solution (n=5) were analyzed to determine the USP method repeatability. Sample contained Benazepril (1 ppm) + imp B, C, D, E, F and G (10 ppm each) in mobile phase.

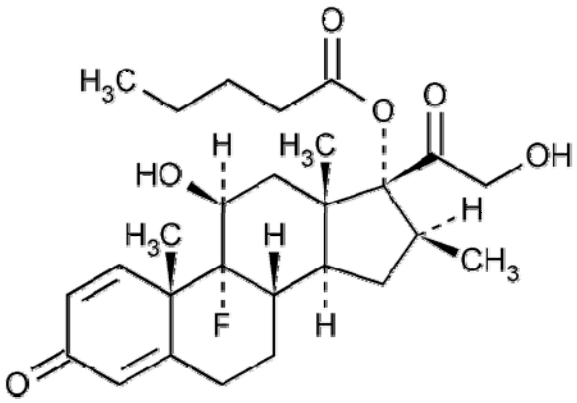
Limit of Detection (LOD) and Limit of Quantitation (LOQ)

No.	Compound	LOD (ppm)	LOQ (ppm)	Curve Equation	Regression Coefficient (R^2)
1	Impurity E	0.44	1.34	y = 17465x - 2100	0.9995
2	Impurity F	0.09	0.27	y = 14795x - 2521.7	0.9996
3	Impurity C	0.15	0.45	y = 10114x - 978.38	0.9998
	Benazepril	-	-		
4	Impurity B	0.24	0.74	y = 3101.4x + 82.818	1.0000
5	Impurity D	0.20	0.60	y=2837.3x - 53.155	0.9998
6	Impurity G	0.20	0.60	y = 2364.5x + 41.812	0.9999

For each of the Benazepril Related Compounds, injections were carried out of at least seven different concentrations from LOQ level to 150 % of standard conc. to determine the linearity of the method.

Betamethasone

USP Method Betamethasone Valerate Assay



Original Manufacturer: -

Original Brand Name: -

Generic Names: Betamethasone dipropionate:
Diprosone, Diprolene, Celestamine and others)
Betamethasone sodium phosphate:
Bentelan, Betamethasone valerate
Betnovate, Celestone and others).

Combination Drugs:

Lotrisone	(Betamethasone and clotrimazole)
Lotriderm	(Betamethasone and clotrimazole)

Betamethasone is a glucocorticoid steroid with anti-inflammatory and immunosuppressive properties. It is applied as a topical cream, ointment, foam, lotion or gel to treat itching.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Betamethasone Valerate

USP34 – NF29 S1

USP Columns

MicroBondapak C18 (Assay)

Equivalent Column:
Purospher®STAR RP-18 endcapped (5 µm) 250x4.0 mm (1.50252.0001)

Optional Scaled Column:
Purospher®STAR RP-18 endcapped (5 µm) 250x3.0 mm (1.50254.0001)

Recommended Solvents and Reagents:

Methanol	for liquid chromatography LiChrosolv®	(1.06018)
Acetonitrile	isocratic grade for liquid chromatography LiChrosolv®	(1.14291)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	(1.15333)
Acetic Acid	Acetic acid (glacial) 100% . Use ACS reagent grade	

USP Standards

Betamethasone Valerate (200 mg)	USP Product Number:	1069007
Beclomethasone Dipropionate (200 mg)	USP Product Number:	1048506

USP Method Betamethasone Valerate Assay

Mobile phase
Prepare a filtered and degassed mixture of acetonitrile and water (3:2).
Make adjustments if necessary (see System Suitability under Chromatography 621).

Internal standard solution
Transfer about 40 mg of beclomethasone dipropionate to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix.

Standard preparation
Transfer about 30 mg of USP Betamethasone Valerate RS, accurately weighed, to a 50-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of Internal standard solution, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Betamethasone Valerate RS per mL.

Assay preparation
Transfer about 60 mg of Betamethasone Valerate, accurately weighed, to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of Internal standard solution, and mix.

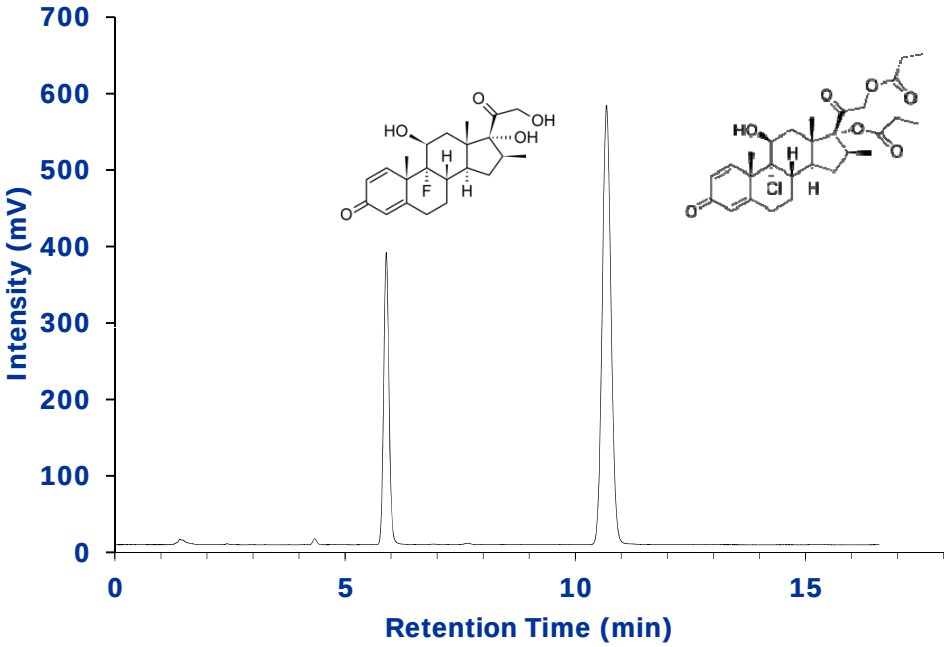
Chromatographic system
The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution, R, between betamethasone valerate and beclomethasone dipropionate is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

USP Method for Betamethasone Valerate Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x4.0 mm	1.50252.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV 254 nm	
Cell:	8 µL	
Flow Rate:	1.2 mL/min	
Mobile Phase (v/v):	Acetonitrile and water 60:40	
Temperature:	Ambient	
Diluent	Methanol with 0.1 % glacial acetic acid	
Sample:	200 ppm Betamethasone Valerate RS and 267 ppm Beclomethasone Dipropionate	
Pressure Drop:	172 Bar (2494 psi)	



Chromatographic Data

No.	Compound	Time (min)	Resolution	Relative Retention Time (RRT)	Tailing Factor (T_{USP})
1	Betamethasone Valerate RS	5.9	-	1.0	1.10
2	Beclomethasone Dipropionate	10.7	17.1	1.8	1.07

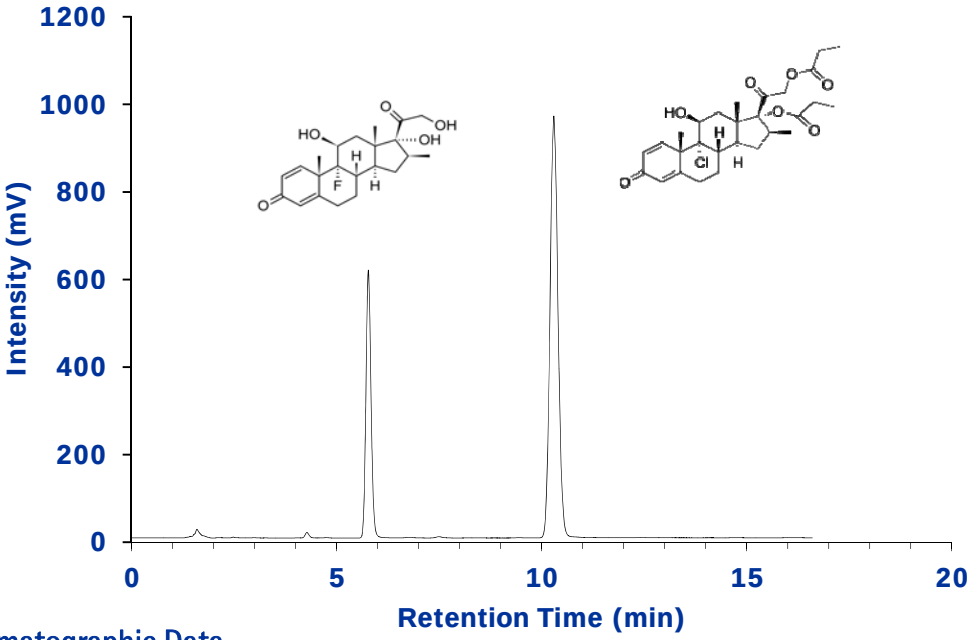
Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Betamethasone Valerate Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x3.0 mm	1.50254.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV 254 nm	
Cell:	8 µL	
Flow Rate:	0.7 mL/min	
Mobile Phase (v/v):	Acetonitrile and water 60:40	
Temperature:	Ambient	
Diluent	Methanol with 0.1 % glacial acetic acid	
Sample:	200 ppm Betamethasone Valerate RS and 267 ppm Beclomethasone Dipropionate	
Pressure Drop:	164 Bar (2378 psi)	



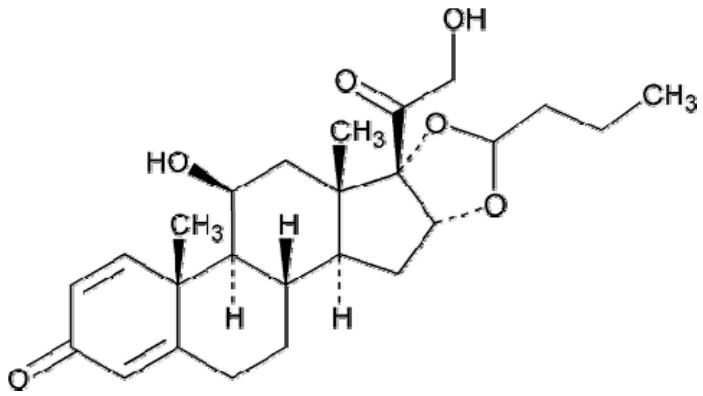
Chromatographic Data

No.	Compound	Time (min)	Resolution	Relative Retention Time (RRT)	Tailing Factor (T_{USP})
1	Betamethasone Valerate RS	5.8	-	1.0	1.12
2	Beclomethasone Dipropionate	10.3	15.5	1.8	1.09

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Budesonide

USP Method Budesonide Assay



Original Manufacturer: AstraZeneca (patent expired)

Original Brand Name: Rhinocort (nasal inhalant)
Pulmicort (oral inhalant)
Entocort (enema or oral capsule)

Generic Names: Budanase AQ, Budate, Budecort , Budez, Budvent, Buovent, Derinide

Combination Drugs: Symbicort (Budesonide and Formoterol)

Budesonide is a glucocorticoid steroid for the treatment of asthma and non-infectious rhinitis . In addition, it is used for Crohn's disease (inflammatory bowel disease).

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Budesonide

USP34 – NF29 S1

USP Columns :

Supelcosil LC-18, 4.6 mm x 15 cm, 5 µm.

ZORBAX SB-C18, 4.6 mm x 15 cm, 3.5 µm.

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm (1.51455.0001)

Recommended Solvents and Reagents:

Acetonitrile	isocratic grade for liquid chromatography LiChrosolv®	(1.14291)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	(1.15333)

Phosphoric Acid	Use ACS reagent grade
Sodium di-hydrogen phosphate	Use ACS reagent grade

USP Standards

Budesonide (200 mg)	USP Product Number:	1078201

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method Budesonide Assay

Mobile phase
Solution A: 3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid.
The pH is 3.2 ± 0.1. Mix Acetonitrile and Solution A (32:68)

Standard solution
Dissolve a quantity of USP Budesonide RS in acetonitrile and dilute quantitatively with Solution A to obtain a solution having a concentration of 0.5 mg/mL, keeping the proportion of acetonitrile in this solution to not more then (NMT) 30%.

Sample solution
Dissolve 25 mg of Budesonide in 15 mL of acetonitrile in a 50-mL volumetric flask, and dilute with Solution A to volume.

Chromatographic system (*See Chromatography 621, System Suitability.*)
Detector: UV 254 nm
Flow rate: 1.5 mL/min
Column: 4.6-mm × 15-cm; 5-µm packing L1
Injection size: 20 µL

System suitability (Sample = Standard solution)
Relative retention time for epimer A is 1.1 with respect to epimer B
Resolution: Not less then (NLT) 1.5 between Budesonide epimer A and B
Column efficiency: NLT 5500 theoretical plates, determined from the Budesonide epimer B peak

Analysis (Samples: Standard solution and Sample solution)
Calculate the percentage of epimer A ($C_{25}H_{34}O_6$) in the portion of Budesonide taken:
Result = $[r_{UA}/(r_{UA} + r_{UB})] \times 100$
r_{UA} = = epimer A peak area from Sample solution
r_{UB} = = epimer B peak area from Sample solution

Calculate the percentage of $C_{25}H_{34}O_6$ in the portion of Budesonide taken:
Result = $[(r_{UA} + r_{UB})/(r_{SA} + r_{SB})] \times (C_S/C_U) \times 100$
r_{UA} = = epimer A peak area from Sample solution
r_{UB} = = epimer B peak area from Sample solution
r_{SA} = = epimer A peak area from Standard solution
r_{SB} = = epimer B peak area from Standard solution
C_S = = concentration of USP Budesonide RS in the Standard solution (mg/mL)
C_U = = concentration of Budesonide in the Sample solution (mg/mL)

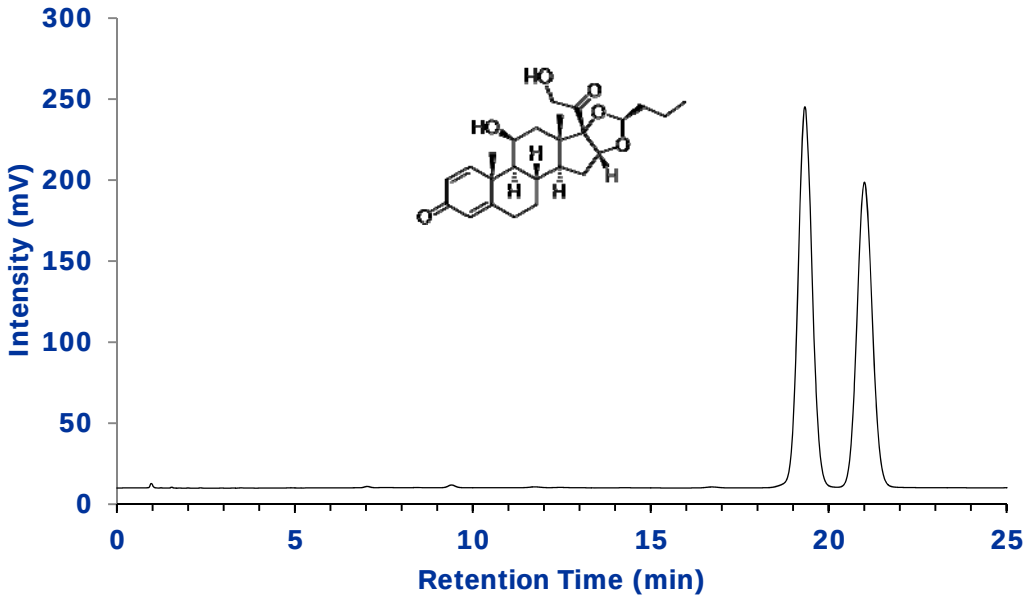
Acceptance criteria
Epimer A: 44.0%–51.0% on the dried basis Both epimers: 98.0%–102.0% on the dried basis

USP Method for Budesonide Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV 254 nm	
Cell:	8 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase (v/v):	Buffer: 3.17 gram of sodium di-hydrogen phosphate in water. Add 0.23 gram of Ortho-phosphoric acid and dilute with water to 1000 ml. pH of solution should be 3.2 +/-0.1 Mix buffer and acetonitrile: 68:32	
Temperature:	Ambient	
Diluent	Solution A (phosphate buffer with an adjusted pH to 3.2 ± 0.1).	
Sample:	500 ppm (0.5 mg/mL) Budesonide	
Pressure Drop:	180 Bar (2610 psi)	



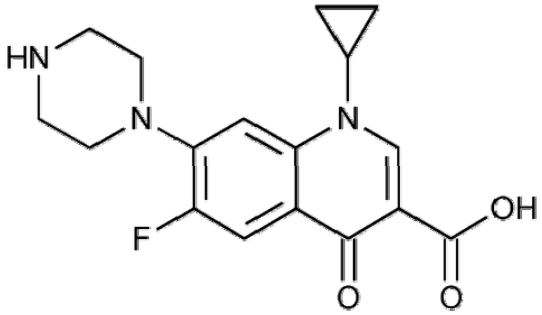
Chromatographic Data

No.	Compound	Time (min)	Resolution	Relative Retention Time (RRT)	Plates (N)
1	Budesonide Epimer B	19.3	-	1.1	10695
2	Budesonide Epimer A	21.0	2.2	1.1	10128

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Ciprofloxacin

USP Method Ciprofloxacin Assay



Original Manufacturer:	Bayer A.G (patent expired)
Original Brand Name:	Ciloxan, Cipro,
Generic Names:	Baycip, Ciprex, Cetraxal, Ciflox, Cipro XR, Cipro XL, Ciproxin, Prociflor, Proquin

Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein.

Ciprofloxacin is marketed worldwide with over three hundred different brand names.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Ciprofloxacin

USP34 – NF29 S1

USP Columns:

Prodigy ODS (3) Assay and Chromatographic purity 4.6 mm x 25 cm, 5 µm, Phenomenex

Equivalent Column:

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

Recommended Solvents and Reagents:

Acetonitrile isocratic grade for liquid chromatography LiChrosolv® (1.14291)

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

Phosphoric Acid Use ACS reagent grade

Triethylamine Use a suitable grade with a content of not less than 99.5%. (8.45061)

USP Standards

Ciprofloxacin (200 mg)	USP Product Number:1134313
Ciprofloxacin Ethylenediamine Analog (25 mg)	USP Product Number:1134324

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method Ciprofloxacin Assay

Buffer solution A: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.

Mobile phase (v/v): Acetonitrile and Solution A (13:87)

Standard solution
Transfer 12.5 mg of USP Ciprofloxacin RS to a 25-mL volumetric flask.
Add 0.1 mL of 7% phosphoric acid, and dilute with Mobile phase to volume.

System suitability stock solution
0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in Mobile phase

System suitability solution
Transfer 1.0 mL of the System suitability stock solution to a 10-mL volumetric flask, and dilute with the Standard solution to volume.

Sample solution
Transfer 25 mg of Ciprofloxacin to a 50-mL volumetric flask. Add 0.2 mL of 7% phosphoric acid, and dilute with Mobile phase to volume.

Chromatographic system *(See Chromatography 621, System Suitability.)*
Detector: UV 278 nm
Column temperature: 30 ± 1
Injection size: 10 µL
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.5 mL/min

System suitability
Samples: Standard solution and System suitability solution
[Note—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are about 0.7 and 1.0, respectively.]

Suitability requirements
Resolution: Not less then (NLT) 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin

Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak

Tailing factor: Not more than (NMT) 2.5 for the ciprofloxacin peak

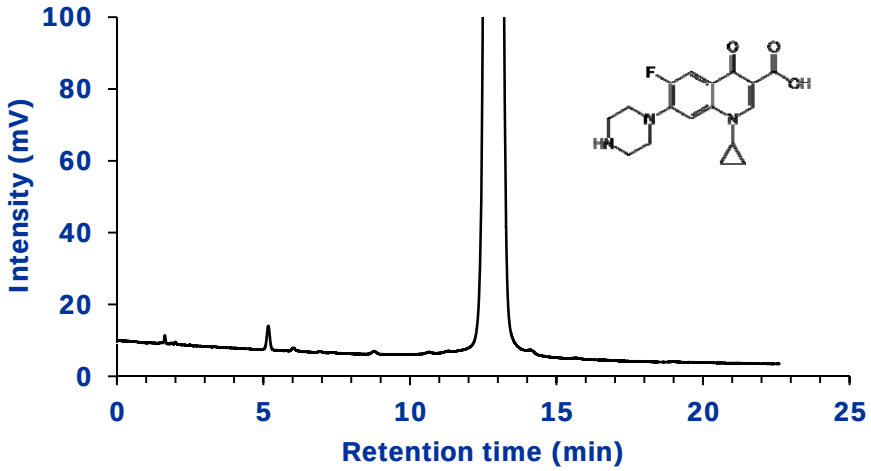
Relative standard deviation: NMT 1.5%

USP Method for Ciprofloxacin Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV 278 nm	
Cell:	8 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase (v/v):	Buffer: 0.025 M phosphoric acid, previously adjusted (with triethylamine) to a pH of 3.0 ± 0.1 Mix buffer and acetonitrile: 87:13	
Temperature:	30° Celsius	
Diluent	mobile phase	
Sample:	500 ppm of Ciprofloxacin	
Pressure Drop:	158 Bar (2290 psi)	



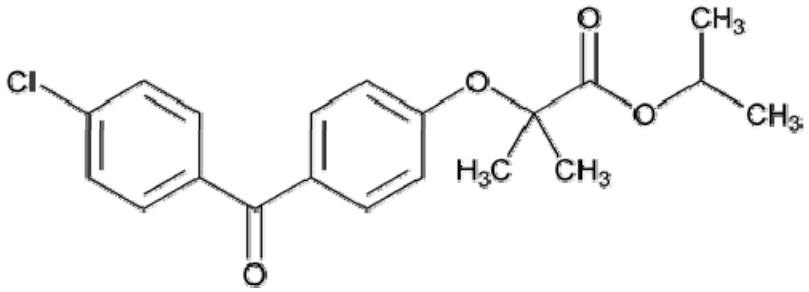
Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Plates (N)
1	Impurity 1	5.2	1.0	-	
2	Impurity 2	6.0	1.4	-	
3	Ciprofloxacin ethylenediamine	8.8	1.0	0.7	13454
4	Ciprofloxacin	12.7	1.8	1.0	8105
5	Impurity 3	14.1	1.2	-	
6	Impurity 4	15.7	1.0	-	

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Fenofibrate

USP Method Fenofibrate RS
USP Method Fenofibrate Assay



Original Manufacturer:	Abbott Laboratories (patent expires 2012)
Original Brand Name:	Tricor and Trilipix
Generic Names:	Lipofen (Kowa Pharmaceuticals America Inc) Lofibra (Teva) Lipanthyl, Lipidil, and Supralip (Solvay Pharmaceutical) and as Fenocor-67, Fenogal, Antara, Golip

Fenofibrate is a drug of the fibrate class. Fenofibrate was developed by Groupe Fournier SA, before it was acquired in 2005 by Solvay Pharmaceutical. In 2009 Solvay Pharmaceutical was acquired by Abbott Laboratories.

Fenofibrate is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease. Fenofibrate reduces both low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as increasing high-density lipoprotein (HDL) levels and reducing triglycerides level.

Fenofibrate is used alone or in conjunction with statins in the treatment of hypercholesterolemia and hypertriglyceridemia.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Fenofibrate

USP34 – NF29 S1

USP Columns:

LiChrospher RP-18e Assay and Related Compounds 4.0 mm x 25 cm, 5 µm, Merck KGaA

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 250x4.0 mm (1.50037.0001)

Optional Scaled Column (narrower inner diameter):

Purospher®STAR RP-18 endcapped (5 µm) 250x3.0 mm (1.50620.0001)

Recommended Solvents and Reagents:

Acetonitrile isocratic grade for liquid chromatography LiChrosolv® (1.14291)

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

Phosphoric Acid Use ACS reagent grade

USP Standards

Fenofibrate (200 mg)	USP Product Number:	1269447
Fenofibrate Related Compound A (25 mg)	USP Product Number:	1269607
Fenofibrate Related Compound B (25 mg)	USP Product Number:	1269618
Fenofibrate Related Compound C (25 mg)	USP Product Number:	1269629

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Fenofibrate Assay

Mobile phase
Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)

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

Sample solution
1 mg/mL of Fenofibrate in Mobile phase

Chromatographic system *(See Chromatography 621, System Suitability.)*
Detector: UV 286 nm
Column: 4.0-mm × 25-cm; packing L1
Injection size: 5 µL
Flow rate: 1.0 mL/min

System suitability
Sample: Standard solution

Suitability requirements
Relative standard deviation: Not more than (NMT) 1.0% for six replicate injections

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of $C_{20}H_{21}ClO_4$ in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution
r_S = peak response from the Standard solution
C_S = concentration of USP Fenofibrate RS in the Standard solution (mg/mL)
C_U = concentration of Fenofibrate in the Sample solution (mg/mL)

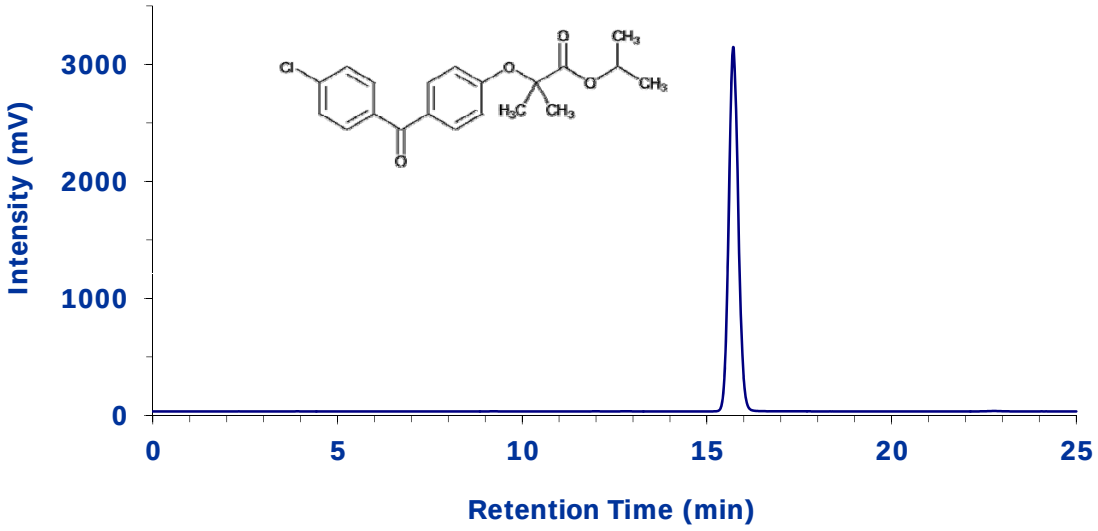
Acceptance criteria:
98.0%–102.0% on the dried basis

USP Method for Fenofibrate Assay

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x4.0 mm	1.50037.0001
Injection:	5 µL	
Detection:	VWR-Hitachi LaChrom Elite, UV@286 nm	
Cell:	13 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Acetonitrile and water acidified with phosphoric acid to a pH of 2.5. Mix water and acetonitrile 30:70.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1.0 mg/mL (1000 ppm) of Fenofibrate	
Pressure Drop:	225 Bar (3263 psi)	



Chromatographic Data

No.	Compound	Time (min)	Plates (N)	Asymmetry (T_{USP})
1	Fenofibrate	15.6	18023	1.07

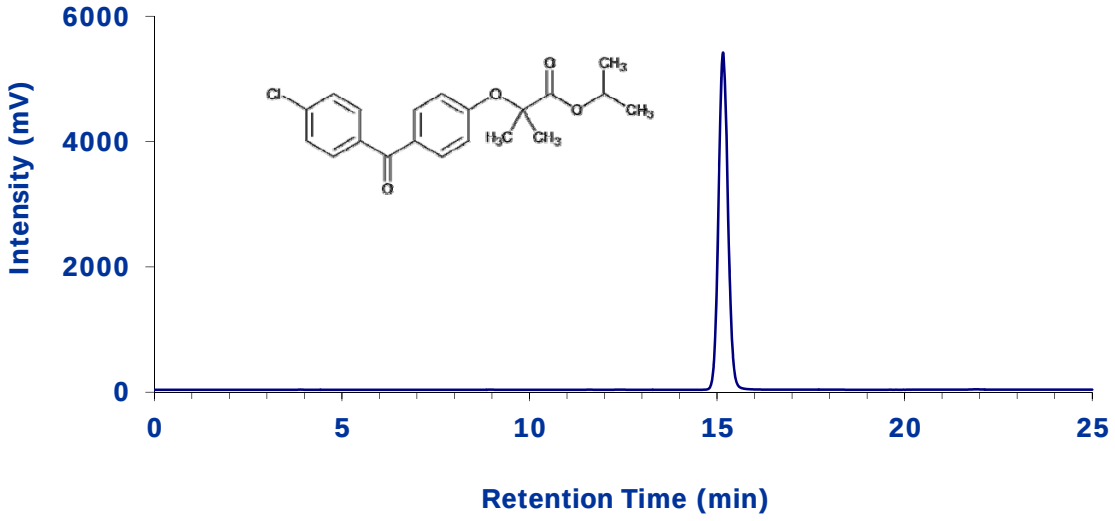
Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Fenofibrate Assay

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x3.0 mm	1.50620.0001
Injection:	5 µL	
Detection:	VWR-Hitachi LaChrom Elite, UV@286 nm	
Cell:	13 µL	
Flow Rate:	0.57 mL/min	
Mobile Phase (v/v):	Acetonitrile and water acidified with phosphoric acid to a pH of 2.5. Mix water and acetonitrile 30:70.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1.0 mg/mL (1000 ppm) of Fenofibrate	
Pressure Drop:	225 Bar (3263 psi)	



Chromatographic Data

No.	Compound	Time (min)	Plates (N)	Asymmetry (T_{USP})
1	Fenofibrate	15.2	16368	1.10

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Fenofibrate RS

Mobile phase
Acetonitrile and water acidified with phosphoric acid to a pH of 2.5 (7:3)

Impurity standard solution
1 µg/mL (1 ppm) each of USP Fenofibrate RS, USP Fenofibrate Related Compound A RS, and USP Fenofibrate Related Compound B RS, and 2 µg/mL (2 ppm) of USP Fenofibrate Related Compound C RS in Mobile phase

Sample solution
1 mg/mL of Fenofibrate in Mobile phase

Chromatographic system *(See Chromatography 621, System Suitability.)*
Detector: UV 286 nm
Column: 4.0-mm × 25-cm; packing L1
Injection size: 20 µL
Flow rate: 1.0 mL/min

System suitability
Sample: Impurity standard solution

Suitability requirements
Resolution: Not less than (NLT) 1.5 between fenofibrate related compound A and fenofibrate RS B

Analysis
(Samples: Impurity standard solution and Sample solution)
Identify the fenofibrate peak and the peaks due to the impurities and degradation products listed in Impurity Table 1. Measure the responses for the major peaks, and calculate the percentage of each of fenofibrate related compound A, fenofibrate related compound B, and fenofibrate related compound C in the portion of Fenofibrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of appropriate fenofibrate related compound from the Sample solution
r_S = peak response of appropriate fenofibrate related compound from the Impurity standard solution
C_S = concentration of appropriate fenofibrate related compound in the Impurity standard solution (µg/mL)
C_U = concentration of Fenofibrate in the Sample solution (µg/mL)

Continued on next page

USP Method for Fenofibrate RS

Analysis

Calculate the percentage of any other impurity in the portion of Fenofibrate taken:

Result = $(r_U/r_S) \times (C_S/C_U) \times 100$

r_U = peak response of each impurity from the Sample solution
r_S = peak response of fenofibrate from the Impurity standard solution
C_S = concentration of fenofibrate in the Impurity standard solution (µg/mL)
C_U = concentration of Fenofibrate in the Sample solution (µg/mL)

Acceptance criteria

Individual impurities: See Impurity Table 1. RRT means relative retention time.

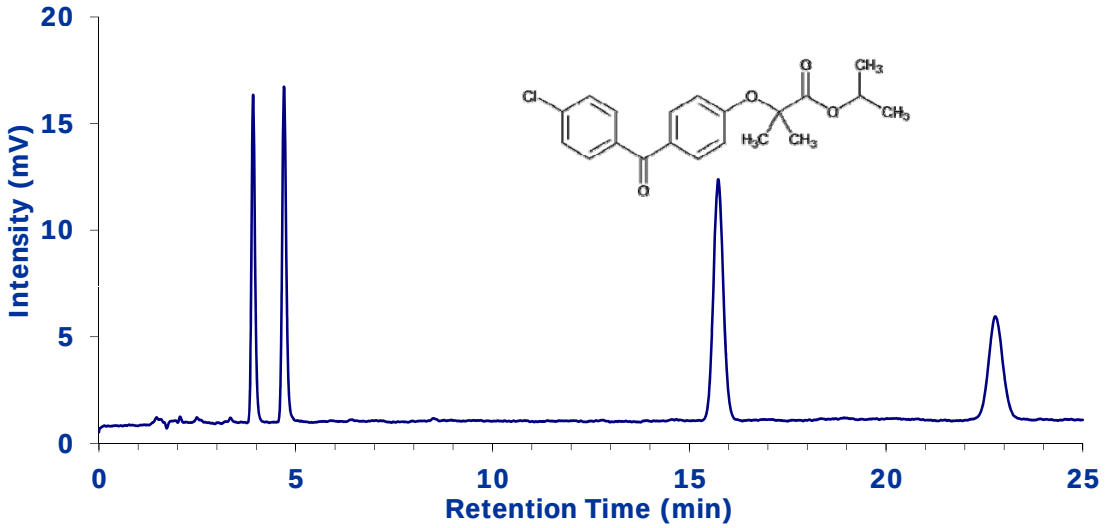
Name	(RRT)	Acceptance Criteria NMT (%)
Fenofibrate related compound A.	0.34	0.1
Fenofibrate related compound B.	0.36	0.1
(3RS)-3-[4-(4-Chlorobenzoyl)phenoxy]butan-2-one	0.50	0.1
Methyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoate	0.65	0.1
Ethyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoate	0.80	0.1
(4-Chlorophenyl)[4-(1-methylethoxy)phenyl]methanone	0.85	0.1
Fenofibrate related compound C.	1.35	0.2
Any other impurity		0.1
Fenofibrate RS A = (4-Chlorophenyl)(4-hydroxyphenyl)methanone		
Fenofibrate RS B = 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid (fenofibric acid)		
Fenofibrate RS C = 1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate		

USP Method for Fenofibrate RS

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x4.0 mm	1.50037.0001
Injection:	20 µL	
Detection:	VWR-Hitachi LaChrom Elite, UV@286 nm	
Cell:	13 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Acetonitrile and water acidified with phosphoric acid to a pH of 2.5. Mix water and acetonitrile 30:70.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1 ppm of Fenofibrate, Fenofibrate RS A and RS B, and 2ppm Fenofibrate RS C	
Pressure Drop:	225 Bar (3263 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Plates (N)	Resolution	Asymmetry (T_{USP})
1	Fenofibrate RS A	3.9	0.25	8919	-	1.2
2	Fenofibrate RS B	4.7	0.30	9719	4.4	1.2
3	**Fenofibrate**	15.7	1.00	17459	33.1	1.1
4	Fenofibrate RS C	22.7	1.45	17947	12.2	1.1

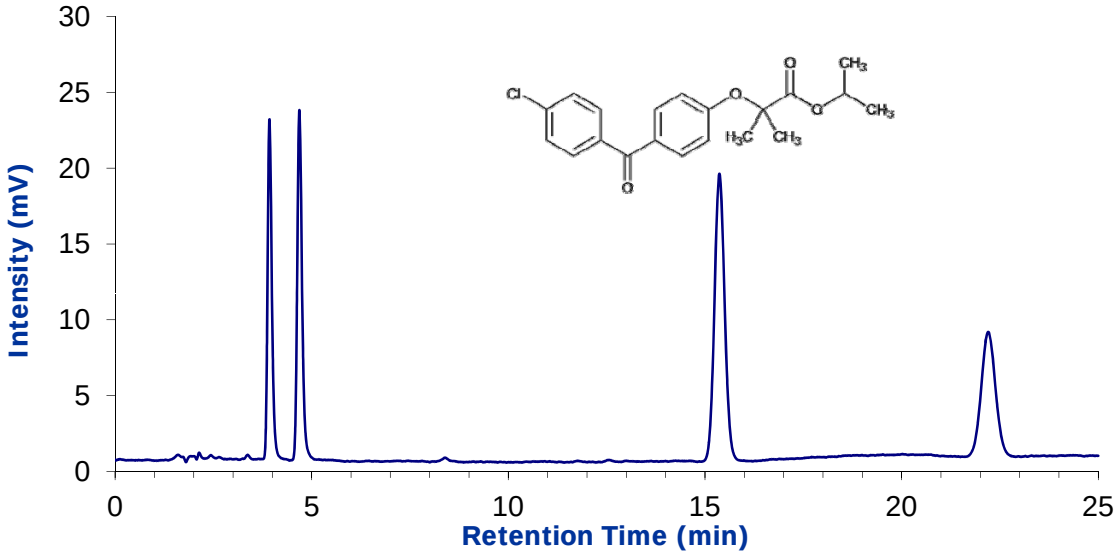
Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Fenofibrate RS

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x3.0 mm	1.50620.0001
Injection:	20 µL	
Detection:	VWR-Hitachi LaChrom Elite, UV@286 nm	
Cell:	13 µL	
Flow Rate:	0.57 mL/min	
Mobile Phase (v/v):	Acetonitrile and water acidified with phosphoric acid to a pH of 2.5. Mix water and acetonitrile 30:70.	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1 ppm of Fenofibrate, Fenofibrate RS A and RS B, and 2ppm Fenofibrate RS C	
Pressure Drop:	179 Bar (2596 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Plates (N)	Resolution	Asymmetry (T_{USP})
1	Fenofibrate RS A	3.9	0.26	6277	-	1.3
2	Fenofibrate RS B	4.7	0.30	7291	3.6	1.3
3	**Fenofibrate**	15.4	1.00	15826	30.2	1.1
4	Fenofibrate RS C	22.2	1.45	16858	11.7	1.1

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Analysis protocol for Fenofibrate

USP Method Repeatability (Purospher®STAR RP-18endcapped (5 µm) 250x4.0 mm)

No.	Compound	Mean Response (Arbitrary Area Units)	Relative Standard Deviation (%)	N
1	Fenofibrate	55472824	0.34	15

USP Method Repeatability (Purospher®STAR RP-18endcapped (5 µm) 250x3.0 mm)

No.	Compound	Mean Response (Arbitrary Area Units)	Relative Standard Deviation (%)	N
1	Fenofibrate	94554999	0.34	15

15 Replicate injections of standard solution (n=15) were analyzed to determine the USP method repeatability. Sample contained 1 mg/mL (1000 ppm) of Fenofibrate in Mobile phase

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

(Purospher®STAR RP-18endcapped (5 µm) 250x3.0 mm)

No.	Compound	LOD (ppm)	LOQ (ppm)	Curve Equation	Regression Coefficient (R^2)
1	Fenofibrate	17.1	51.8	y = 53940x + 815390	0.9998

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

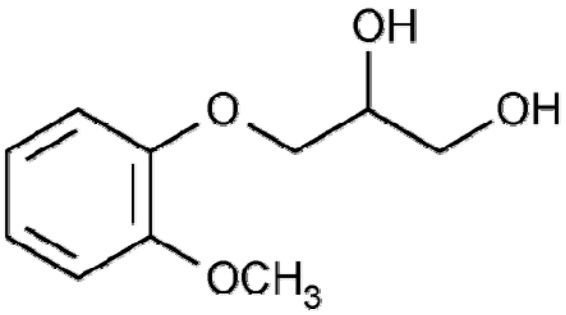
(Purospher®STAR RP-18endcapped (5 µm) 250x3.0 mm)

No.	Compound	LOD (ppm)	LOQ (ppm)	Curve Equation	Regression Coefficient (R^2)
1	Fenofibrate	60.4	183	y = 86321x + 7E+06	0.9976

Injection of at least seven different conc. from LOQ level to 150 % of standard concentration 1 mg/mL (1000 ppm) to determine the linearity of the method.

Guaifenesin

USP Method Guaifenesin Assay



Original Manufacturer: FDA approved in 1952

Brand Name: Humibid, Humibid LA, Robitussin, Organidin NR, Fenesin, Mucinex, Cheratussin, Benylin, DayQuil Mucous Control, Meltus, and Bidex 400.

Guaifenesin or guaiphenesin, also glyceryl guaiacolate, is an expectorant drug and usually taken by mouth that promotes elimination of mucus from the lungs. Hence it assist the bringing up (expectoration) of phlegm from the airways in acute respiratory tract infections.

Guaifenesin is sold as pills or syrups under many brand names. Single-ingredient formulations of guaifenesin are available, and it is also included in many other over-the-counter cough and cold remedy combinations (usually in conjunction with dextromethorphan and/or pseudoephedrine or phenylephrine and/or acetaminophen).

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Guaifenesin

USP34 – NF29 S1

USP Columns:

Nucleosil C18 Assay and Chromatographic purity 4.6 mm x 25 cm, 5 µm

Equivalent Column:

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

Recommended Solvents and Reagents:

Acetonitrile gradient grade for liquid chromatography LiChrosolv® (1.00030)

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

Acetic Acid Acetic acid (glacial) 100%. Use ACS reagent grade

USP Standards

Guaifenesin (200 mg) USP Product Number:1301007
Guaiacol (1 g) USP Product Number:1300004

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method Guaifenisin Assay

Assay
Solution A: Prepare a mixture of water and glacial acetic acid (990:10).
Solution B: Use acetonitrile.

Mobile phase
Use variable mixtures of Solution A and Solution B as directed for Chromatographic system.
Make adjustments if necessary (*see System Suitability under Chromatography 621*).

Resolution solution
Prepare a solution in Solution B containing about 0.5 mg of USP Guaifenesin RS and 0.02 mg of USP Guaiacol RS in each mL.

Standard preparation
Prepare a solution of USP Guaifenesin RS in Solution B with known concentration of about 0.5 mg per mL.

Assay preparation
Transfer about 25 mg of Guaifenesin, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with Solution B to volume, and mix.

Chromatographic system (*see Chromatography 621*)
The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Gradient programmed as follows:

Time (min)	Solution A (%)	Solution B (%)	Elution
0-32	80→50	20→50	Linear gradient
32-35	50→80	50→20	Linear gradient

Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.9 for guaifenesin isomer, 1.0 for guaifenesin, and 1.3 for guaiacol; and the resolution, R, between guaifenesin and guaiacol is not less than 3.

Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 1.0%.

USP Method Guaifenisin Assay

Procedure

Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses.
Calculate the quantity of $C_{10}H_{14}O_4$ in the portion of Guaifenesin taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Guaifenesin RS in the Standard preparation; and r_U and r_S are the peak areas obtained from the Assay preparation and the Standard preparation, respectively.
Calculate the percentage of $C_{10}H_{14}O_4$ in the portion of Guaifenesin taken.
To this value, add the percentage of guaifenesin isomer found in the test for Chromatographic purity.

Solution A, Solution B, and Mobile phase *(Proceed as directed in the Assay.)*

Chromatographic system (*Proceed as directed in the Assay*)
To evaluate the system suitability requirements, use the Resolution solution and the Standard preparation prepared as directed in the Assay.

Test solution
Dissolve about 20 mg of Guaifenesin in 10 mL of Solution B.

Diluted test solution
Transfer 1.0 mL of Test solution to a 100-mL volumetric flask, dilute with Solution B to volume, and mix.

Separately inject equal volumes (about 10 µL) of the Test solution and the Diluted test solution into the chromatograph, record the chromatograms, and measure the areas for the major peaks. All of the peaks are baseline resolved. Calculate the percentage of each impurity in the portion of Guaifenesin taken by the formula:

$$F(r_i / r_S)$$

in which F is a response factor equal to 0.63 for the guaiacol peak, having a relative retention time of 1.4, and 1.0 for all other impurities; r_i is the area of each peak, other than that of the main guaifenesin peak, obtained from the Test solution; and r_S is the area of the main peak obtained from the Diluted test solution: not more than 1.5% of 2-(2-methoxyphenoxy)-1,3-propanediol (guaifenesin isomer), the peak for which occurs at a relative retention time of about 0.9, is found; not more than 0.03% of guaiacol is found; not more than 0.5% of any other individual impurity is found; and not more than 1.0% of total impurities, excluding guaifenesin isomer and guaiacol, is found.

USP Method for Guaifenisin Assay

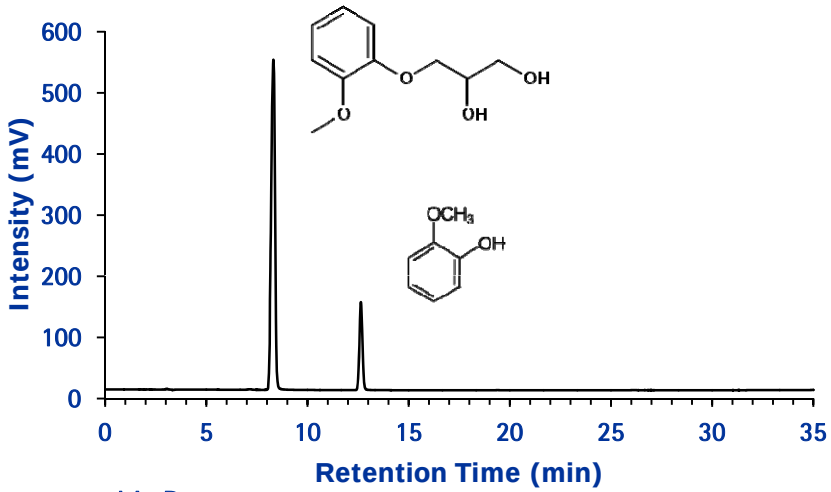
Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV@276 nm	
Cell:	8 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Glacial acetic acid and water (10:990) Solution B: Acetonitrile	
Gradient:	See Table:	

Time (min)	Solution A (%)	Solution B (%)	Elution
0-32	80→50	20→50	Linear gradient
32-35	50→80	50→20	Linear gradient

Temperature:	30° Celsius
Diluent	mobile phase
Sample:	500 ppm (0.5 mg/mL) Guaiphenesin and 20 ppm (0.02 mg/mL) Guaiacol
Pressure Drop:	142 Bar (2059 psi)



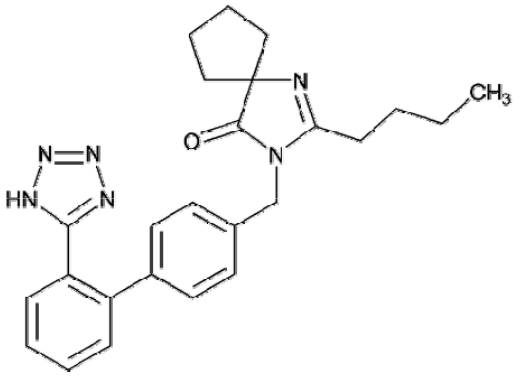
Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Guaifenesin beta isomer	7.2	1.0	0.9	
2	Guaifenesin	8.3	1.0	1.0	
3	Guaiacol	12.6	1.1	1.5	4.8
4	Impurity 1	19.4	1.1		
5	Impurity 2	26.1	1.3		

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Irbesartan

USP Method Irbesartan Assay



Original Manufacturer: Sanofi-Aventis (patent expire March 2012)

Original Brand Name: Avapro, Aprovel, Karvea

Combination Drugs: Irda, CoIrda, CoAprovel, Karvezide, Avalide, Avapro HCT (Irbesartan and Hydrochlorothiazide)

Irbesartan is an angiotensin II receptor antagonist used mainly for the treatment of hypertension. It is jointly marketed by Sanofi-Aventis and Bristol-Myers Squibb.

Irbesartan is also available in a combination formulation with a low dose thiazide diuretic, invariably hydrochlorothiazide, to achieve an additive antihypertensive effect.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Irbesartan

USP34 – NF29 S1

USP Columns:

Nucleosil C18 Assay and Related Compounds 4.0 mm x 25 cm, 7 µm, Macherey-Nagel.

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 250x4.0 mm (1.50252.0001)

Optional Scaled Column 1:

Purospher®STAR RP-18 endcapped (5 µm) 250x3.0 mm (1.50254.0001)

Optional Scaled Column 2:

Purospher®STAR RP-18 endcapped (3 µm) 125x3.0 mm (1.50175.0001)

Recommended Solvents and Reagents:

Methanol for liquid chromatography LiChrosolv® (1.06018)

Acetonitrile isocratic grade for liquid chromatography LiChrosolv® (1.14291)

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

Triethylamine Use a suitable grade with a content of not less than 99.5%. (8.45061)
Phosphoric Acid Use ACS reagent grade

USP Standards

Irbesartan (200 mg)	USP Product Number:	1347700
Irbesartan Related Compound A (25 mg)	USP Product Number:	1347711

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method Irbesartan Assay

pH 3.2 Phosphate buffer
Mix 5.5 mL of phosphoric acid with about 950 mL of water, and adjust pH to 3.2 with triethylamine.

Mobile phase
Prepare a filtered and degassed mixture of pH 3.2 phosphate buffer and acetonitrile (67:33).
Make adjustments if necessary (*see System Suitability under Chromatography 621*).

System suitability solution
Dissolve accurately weighed quantities of USP Irbesartan RS and USP Irbesartan Related Compound A RS in methanol to obtain a solution having a known concentration of about 0.05 mg per mL of each USP Reference Standard.

Standard preparation
Dissolve an accurately weighed quantity of USP Irbesartan RS in methanol to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation
Transfer about 50 mg of Irbesartan, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (*see Chromatography 621*)
The liquid chromatograph is equipped with a 220-nm detector and a 4.0-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure:

the relative retention times are about 0.8 for Irbesartan related compound A and 1.0 for Irbesartan
the resolution, R, between Irbesartan and Irbesartan related compound A is not less than 2.0.

Chromatograph the Standard preparation, and record the peak response as directed for Procedure: the standard deviation for replicate injections is not more than 1.0%.

Procedure
Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of $C_{25}H_{28}N_6O$ in the portion of Irbesartan taken by the formula:

$100C(r_U/r_S)$

in which C is the concentration, in mg per mL, of USP Irbesartan RS in the Standard preparation; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method Irbesartan RS

pH 3.2 Phosphate buffer and Mobile phase (*Proceed as directed in the Assay.*)

Standard solution
Prepare as directed for the System suitability solution in the Assay.

Test solution
Dissolve an accurately weighed quantity of Irbesartan in methanol to obtain a solution having a known concentration of about 1 mg per mL.

Chromatographic system *(see Chromatography 621)*
Proceed as directed in the Assay. Chromatograph the Standard solution and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure
Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the area for the Irbesartan related compound A peak. Calculate the percentage of Irbesartan related compound A in the portion of Irbesartan taken by the formula:

$$100(C_S/C_T)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Irbesartan Related Compound A RS in the Standard solution; C_T is the concentration, in mg per mL, of Irbesartan in the Test solution; r_U is the peak response for Irbesartan related compound A obtained from the Test solution; and r_S is the peak response for Irbesartan related compound A obtained from the Standard solution.

Calculate the percentage of other impurities in the portion of Irbesartan taken by the formula:

$$100(C_S/C_T)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of USP Irbesartan RS in the Standard solution; C_T is the concentration, in mg per mL, of Irbesartan in the Test solution; and r_U and r_S are the peak responses for each of the other impurities and USP Irbesartan RS obtained from the Test solution and the Standard solution, respectively:

- not more than 0.2% of Irbesartan related compound A is found
- not more than 0.1% of any other impurity is found
- not more than 0.5% of total impurities is found

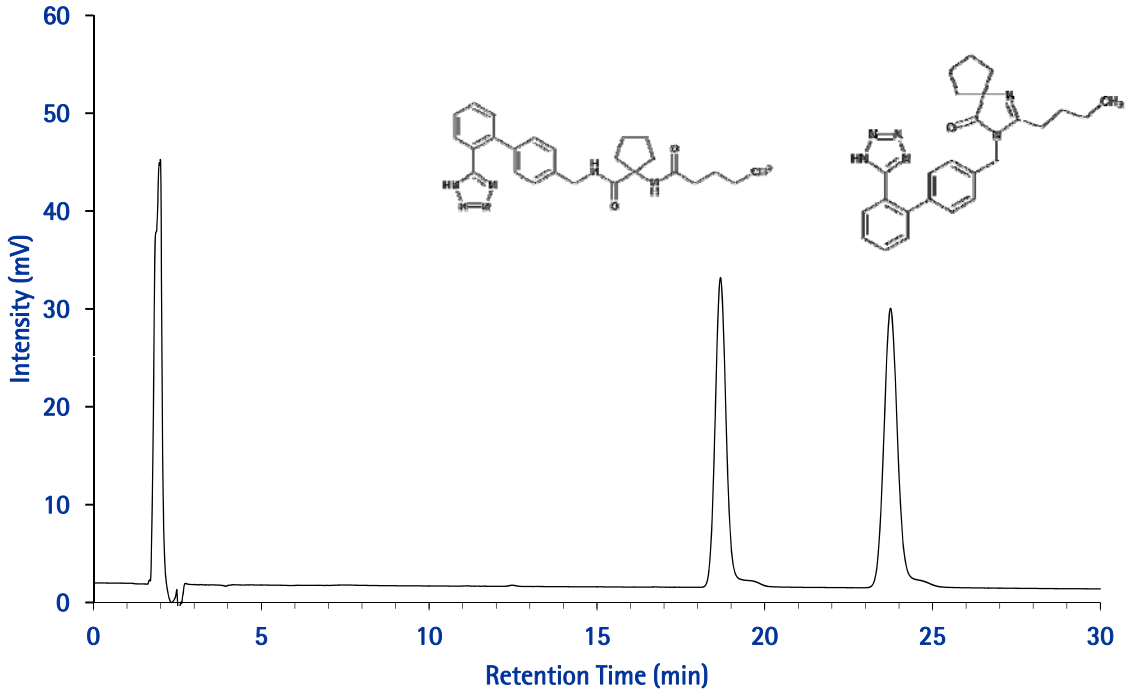
HPLC Application note 2034
USP Method Irbesartan Assay – scaled to narrow id column

USP Method for Irbesartan Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x3.0 mm	1.50254.0001
Injection:	6 µL	
Detection:	Shimadzu Prominence 2010, UV 220 nm	
Cell:	semi-micro cell 2.5 µL	
Flow Rate:	0.6 mL/min	
Mobile Phase (v/v):	Buffer: Mix 5.5 mL of phosphoric acid with about 950 mL of water, and adjust pH to 3.2 with triethylamine. Prepare a filtered and degassed mixture of pH 3.2 phosphate buffer and acetonitrile (67:33).	
Temperature:	Ambient	
Diluent	Methanol	
Sample:	50 ppm (0.05 mg/mL) of each Irbesartan and Irbesartan RS A (SST solution)	
Pressure Drop:	184 Bar (2650 psi)	



Chromatographic Data

No.	Compound	Time (min)	Resolution	Relative Retention Time (RRT)	Tailing Factor (T_{USP})
1	Irbesartan RS A	18.7	-	0.8	1.12
2	Irbesartan	23.7	7.6	1.0	1.13

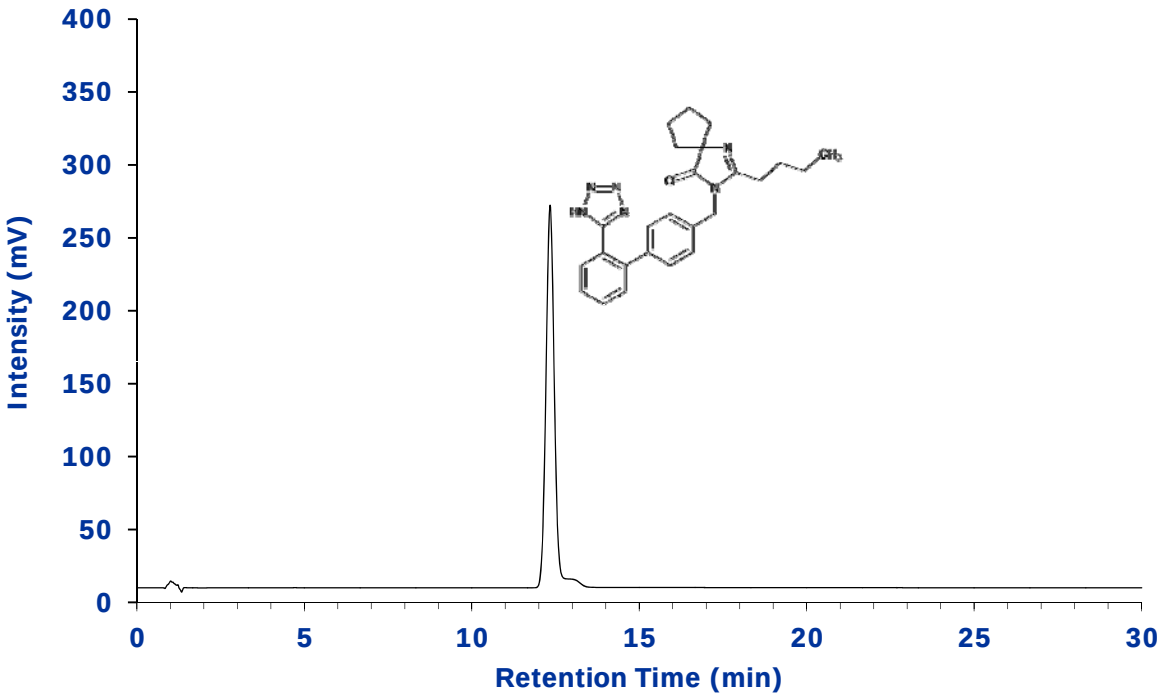
Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Irbesartan Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (3 µm) 125x3.0 mm	1.50175.0001
Injection:	3 µL	
Detection:	Shimadzu Prominence 2010, UV 220 nm	
Cell:	semi-micro cell 2.5 µL	
Flow Rate:	0.6 mL/min	
Mobile Phase (v/v):	Buffer: Mix 5.5 mL of phosphoric acid with about 950 mL of water, and adjust pH to 3.2 with triethylamine. Prepare a filtered and degassed mixture of pH 3.2 phosphate buffer and acetonitrile (67:33).	
Temperature:	Ambient	
Diluent	methanol	
Sample:	500 ppm (0.05 mg/mL) of Irbesartan (Assay solution)	
Pressure Drop:	204 Bar (2938 psi)	



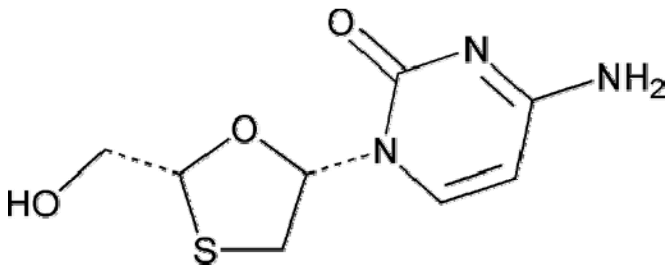
Chromatographic Data

No.	Compound	Time (min)	Resolution	Relative Retention Time (RRT)	Tailing Factor (T_{USP})
1	Irbesartan RS A	-	-	-	-
2	Irbesartan	12.3	-	1.0	1.08

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Lamivudine

USP Method Lamivudine RS



Original Manufacturer:	GlaxoSmithKline (GSK) patent expiry: 2010 (US) and 2011 (Europe)
Brand Name:	Zeffix, Heptovir, Epivir, and Epivir-HBV
Generics:	Lamivir HBV (Cipla)
Combination Drugs:	Combivir (with zidovudine); Epzicom/Kivexa (with abacavir); Trizivir (with zidovudine and abacavir)

Lamivudine (2',3'-dideoxy-3'-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI).

Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Lamivudine

USP34 – NF29 S1

USP Columns:

Hypersil BDS C-18 Assay and Chromatographic purity 4.6-mm x 25-cm., Thermo

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

Ammonium Acetate Use ACS reagent grade.

Acetic Acid Acetic acid (glacial) 100%. Use ACS reagent grade.

USP Standards

Lamivudine (200 mg)	USP Product Number:1356836
Lamivudine Resolution Mixture A (10 mg)	USP Product Number:1356847
Lamivudine Resolution Mixture B (10 mg)	USP Product Number: 1356858

USP Method Lamivudine Assay

Buffer - 0.025 M Ammonium acetate solution:

Transfer about 1.9 g of ammonium acetate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with acetic acid to a pH of 3.8 ± 0.2, dilute with water to volume, and mix.

Mobile phase

Prepare a filtered and degassed mixture of 0.025 M Ammonium acetate solution and methanol (95:5). Make adjustments if necessary (see System Suitability under Chromatography 621).

System suitability solution

Dissolve an accurately weighed quantity of USP Lamivudine Resolution Mixture B RS in Mobile phase to obtain a solution having a known concentration of about 0.25 mg per mL.

Standard preparation

Dissolve an accurately weighed quantity of USP Lamivudine RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.25 mg per mL.

Assay preparation

Transfer about 25 mg of Lamivudine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 277-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 35° C.

Chromatograph the System suitability solution, and record the peak responses as directed for Procedure:

Resolution, R, between lamivudine and lamivudine diastereomer is not less than 1.5.
Relative retention times are about 1.0 for lamivudine and 0.9 for lamivudine diastereomer.

Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

USP Method Lamivudine RS

Procedure

Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the lamivudine peaks. Calculate the quantity, in mg, of $C_8H_{11}N_3O_3S$ in the portion of Lamivudine taken by the formula:

$100C(rU / rS)$

in which C is the concentration, in mg per mL, of USP Lamivudine RS in the Standard preparation; and rU and rS are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Chromatographic purity

0.025 M Ammonium acetate solution, Mobile phase, System suitability solution, and Chromatographic system, proceed as directed in the Assay.

Salicylic acid solution

Dissolve an accurately weighed quantity of salicylic acid in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a concentration of about 0.625 µg/mL.

Standard solution

Use the Standard preparation, prepared as directed in the Assay.

Test solution

Use the Assay preparation.

Procedure

Separately inject equal volumes (about 10 µL) of Salicylic acid solution and the Test solution into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of salicylic acid in the portion of Lamivudine taken by the formula:

$(10C/W)(r_U / r_S)$

in which C is the concentration, in µg per mL, of salicylic acid in the Salicylic acid solution; W is the weight, in mg, of Lamivudine taken for the Test solution; and r_U and r_S are the salicylic acid peak responses obtained from the Test solution and the Salicylic acid solution, respectively. Calculate the percentage of other individual impurities in the portion of Lamivudine taken by the formula:

$100(r_i / r_s)$

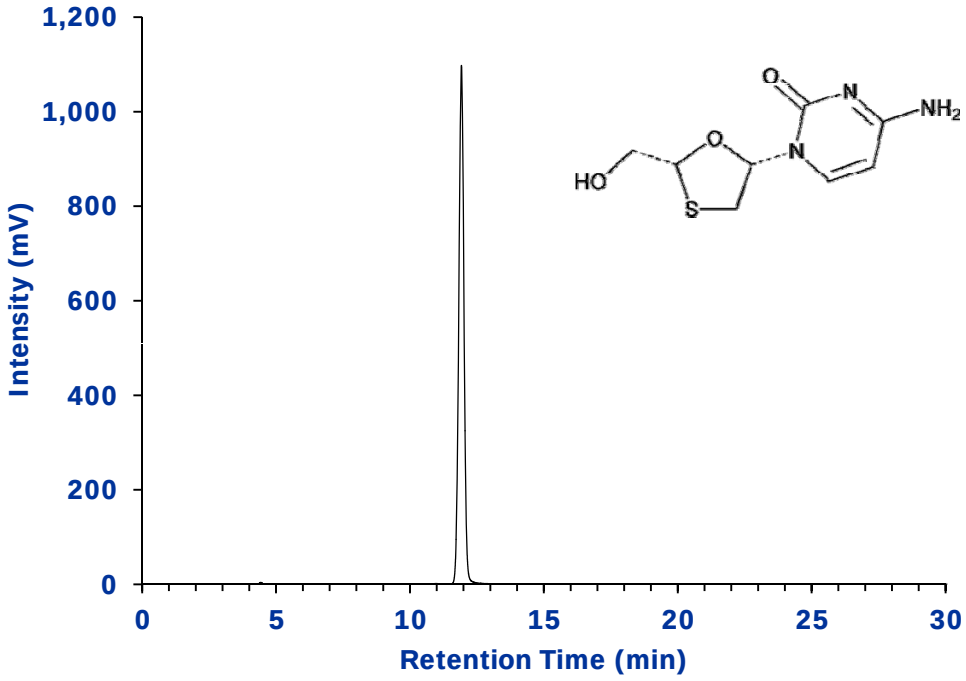
in which r_i is the peak response for each impurity other than salicylic acid obtained from the Test solution; and r_s is the sum of the responses for all the peaks: not more than 0.3% for any peak at a relative retention time of about 0.4 is found; not more than 0.2% for any peak at a relative retention time of about 0.9 is found; not more than 0.1% of salicylic acid is found; not more than 0.1% of any other individual impurity is found; and not more than 0.6% of total impurities is found.

USP Method for Lamivudine Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV@277 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 0.025 M Ammonium acetate solution, with pH adjusted to 3.8 ± 0.2 with acetic acid Mix buffer and methanol 95:5.	
Temperature:	35° Celsius	
Diluent	mobile phase	
Sample:	250 ppm (0.25 mg/mL) Lamivudine	
Pressure Drop:	134 Bar (1943 psi)	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Lamivudine	11.9	1.0	-	-

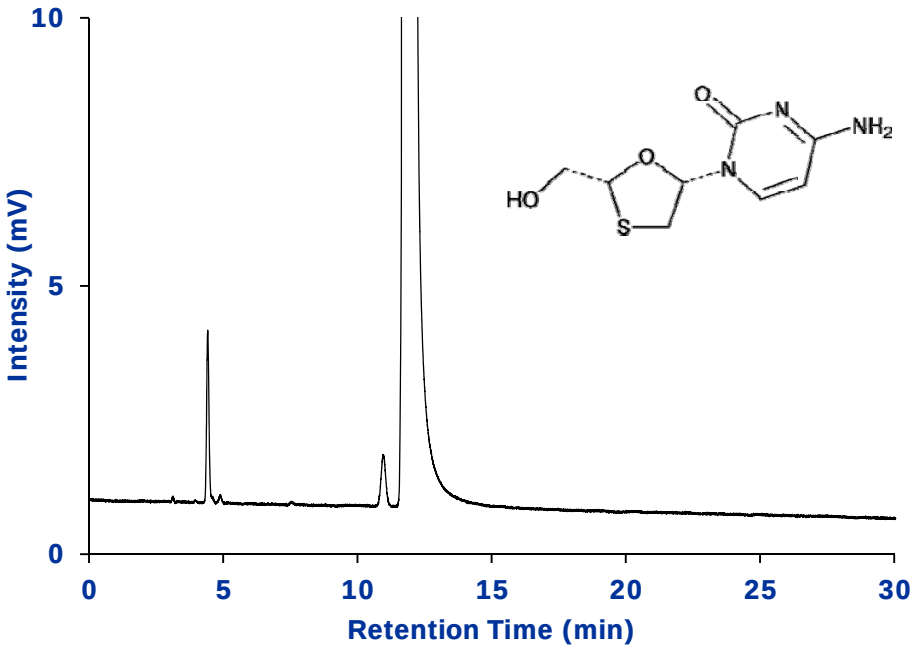
Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Lamivudine RS

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV@277 nm	
Cell:	8 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 0.025 M Ammonium acetate solution, with pH adjusted to 3.8 ± 0.2 with acetic acid Mix buffer and methanol 95:5.	
Temperature:	35° Celsius	
Diluent	mobile phase	
Sample:	250 ppm (0.25 mg/mL) Lamivudine and traces of lamivudine diastereomer	
Pressure Drop:	134 Bar (1943 psi)	



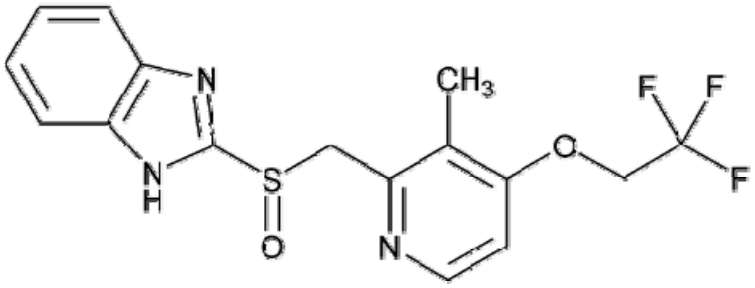
Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Impurity	4.4	1.1	0.4	
2	Lamivudine diastereomer	11.0	1.0	0.9	
3	Lamivudine	11.9	1.0	1.0	2.9

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Lansoprazole

USP Method Lansoprazole RS



Original Manufacturer: Novartis (patent expired November 2009)

Brand Name: Prevacid, Helicid, Zoton, Inhibitol, Monolitum, Agopton, Digest, Duogast, Gastrolan, Lanciprol, Lansazol, Lansobene, Lansoloc, Lansoprazol, Lansoptol, Lansor, Lansox, Lanston LFDT, Lenzo, Lanzol, Lanzotec, Lanzul, Lanton, Lanzo, Lanzor, Lanzostad, Laprazol, Limpidex, Ogast, OgastORO, Ogastro, Prosogan, Prosogan FD, pro-ulco, Refluxon, SOLOX, Takepron, Zolt,

Lansoprazole is a proton-pump inhibitor (PPI) in the same pharmacologic class as omeprazole, and prevents the stomach from producing gastric acid.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Lansoprazole

USP34 – NF29 S1

USP Columns:

YMC-Pack AQ-302 C18 Chromatographic purity 4.6-mm x 15-cm, 5 µm.

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm (1.51455.0001)

Recommended Solvents and Reagents:

Acetonitrile gradient grade for liquid chromatography LiChrosolv® (1.00030)

Methanol for liquid chromatography LiChrosolv® (1.06018)

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

Triethylamine: Use a suitable grade with a content of not less than 99.5%. (8.45061)
Phosphoric Acid: Use ACS reagent grade
Sodium Hydroxide :Use ACS reagent grade

USP Standards

Lansoprazole (150 mg)	USP Product Number:1356916
Lansoprazole Related Compound A (25 mg)	USP Product Number:1356927
Lansoprazole Related Compound B (25 mg)	USP Product Number:1356931

USP Method Lansoprazole

Chromatographic purity

[Store and inject the lansoprazole solutions at or below 5°C using a cooled autosampler. The solutions are stable for about 24 hours when stored at 5°C]

Solution A: water.

Solution B: Prepare a filtered and degassed mixture of acetonitrile, water, and triethylamine (160:40:1). Adjust with phosphoric acid to a pH of 7.0.

Diluent:

Prepare a mixture of 0.1 N sodium hydroxide solution and methanol (75:25).

Blank solution

Prepare a mixture of Diluent and methanol (9:1).

Mobile phase

Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary (*see System Suitability under Chromatography 621*).

Resolution solution

Dissolve 5 mg each of USP Lansoprazole RS and USP Lansoprazole Related Compound A RS in 200 mL of methanol. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with Diluent to volume, and mix.

System suitability solution

Dissolve a suitable quantity of USP Lansoprazole Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.025 mg per mL. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with Diluent to volume, and mix.

Standard solution

Dissolve an accurately weighed quantity of USP Lansoprazole RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 25 µg per mL. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with Diluent to volume, and mix. The final concentration of the Standard solution is about 2.5 µg per mL.

Test solution

Transfer about 125 mg of Lansoprazole, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 1 mL of this solution into a 10-mL volumetric flask, and dilute with Diluent to volume.

USP Method Lansoprazole

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 285-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 0.8 mL per minute. Elution profile as follows.

Time (min)	Solution A (%)	Solution B (%)	Elution
0-40	90→20	10→80	Linear gradient
40-50	20	80	Isocratic
50-51	20→90	80→10	Linear gradient
51-60	90	10	Isocratic

Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the *resolution, R, between lansoprazole and lansoprazole related compound A is not less than 6.*

Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 3%.

Name	Rel. Response Factor (F)	Approx. Rel. Retention Time	Limit (%)
Lansoprazole RS A	0.82	1.1	0.4
Lansoprazole N-oxide2	1.3	0.8	0.1
Lansoprazole RS B	0.79	1.2	0.1
Other individual impurity	1.00	-	0.1
Lansoprazole RS A = Lansoprazole sulfone = (2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole)			
Lansoprazole N-oxide = [[(1H-benzimidazole-2-yl)sulfinyl]methyl]-3-methyl-4-(2,2,2-trifluoroethoxy)-pyridine 1-oxide			
Lansoprazole RS B=2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl]methyl]sulfanyl]-1H-benzimidazole			

Procedure

Separately inject equal volumes (about 40 µL) of the Blank solution, the Standard solution and the Test solution into the chromatograph, record the chromatograms, and identify the lansoprazole peak and the peaks due to the impurities listed in Table 1. Measure the areas for the major peaks, excluding the peaks obtained from the Blank solution. Calculate the percentage of each impurity in the portion of Lansoprazole taken by the formula: $100 \times 0.001(1/F)(C_S/C_T)(r_i/r_S)$

in which F is the relative response factor for each impurity peak (see Table 1 for values); 0.001 is the conversion factor from µg per mL to mg per mL; C_S is the concentration, in µg per mL, of USP Lansoprazole RS in the Standard solution; C_T is the concentration, in mg per mL, of Lansoprazole in the Test solution; r_i is the peak response for each impurity obtained from the Test solution; and r_S is the peak response for lansoprazole obtained from the Standard solution: In addition to not exceeding the limits for impurities in Table 1, not more than 0.6% of total impurities is found. Disregard any peak below 0.05%.

USP Method Lansoprazole Assay

Diluent

Prepare a mixture of water, acetonitrile, and triethylamine (60:40:1), and adjust with phosphoric acid to a pH of 10.0.

Mobile phase

Prepare a filtered and degassed mixture of water, acetonitrile, and triethylamine (60:40:1). Adjust with phosphoric acid to a pH of 7.0. Make adjustments if necessary (*see SST under Chromatography 621*).

Resolution solution

Dissolve suitable quantities of USP Lansoprazole RS and USP Lansoprazole Related Compound A RS in Diluent to obtain a solution containing about 0.1 mg of each per mL.

Internal standard solution

Dissolve an accurately weighed quantity of 4¢-ethoxyacetophenone in Diluent to obtain a solution having a known concentration of about 2.5 mg per mL.

Standard preparation

Dissolve an accurately weighed quantity of USP Lansoprazole RS in Internal standard solution to obtain a solution having a known concentration of about 5.0 mg per mL. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with Diluent to volume, and mix.

Assay preparation

Transfer about 50 mg of Lansoprazole, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with Internal standard solution to volume, and mix. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, dilute with Diluent to volume, and mix.

Chromatographic system *(see Chromatography 621)*

The liquid chromatograph is equipped with a 285-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the resolution, R, between lansoprazole and lansoprazole related compound A is not less than 5. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 1.0%.

Procedure

Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of $C_{16}H_{14}F_3N_3O_2S$ in the portion of Lansoprazole taken by the formula:

$$100(C_S/C_U)(R_U/R_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of lansoprazole in the Standard preparation and the Assay preparation, respectively; and R_U and R_S are the peak response ratios obtained from the Assay preparation and the Standard preparation, respectively.

USP Method for Lansoprazole Assay

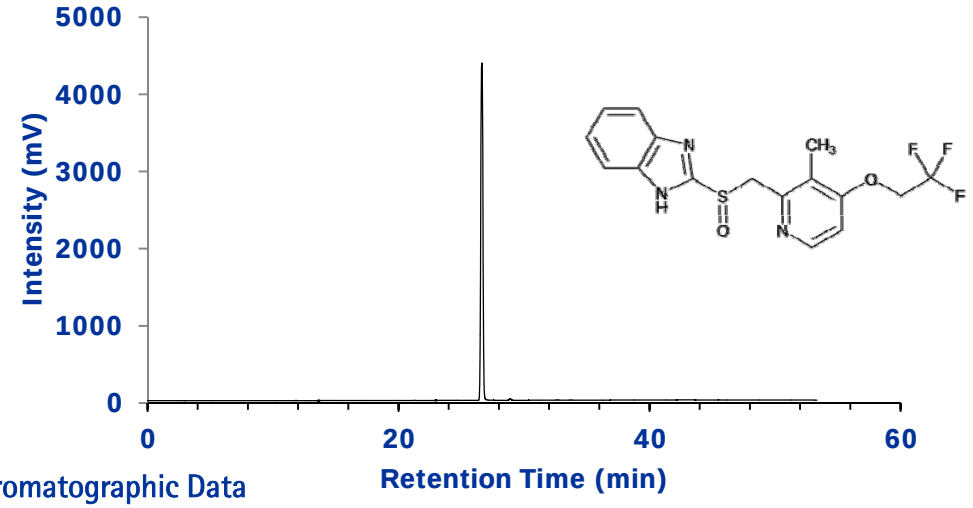
Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	40 µL	
Detection:	Shimadzu Prominence 2010, UV 285 nm	
Cell:	8 µL	
Flow Rate:	0.8 mL/min	
Mobile Phase (v/v):	Solution A: 100% Water Solution B: Acetonitrile, water, and triethylamine (160:40:1)with a pH of 7.0	
Gradient	See Table	

Time (min)	Solution A (%)	Solution B (%)	Elution
0-40	90→20	10→80	Linear gradient
40-50	20	80	Isocratic
50-51	20→90	80→10	Linear gradient
51-60	90	10	Isocratic

Temperature:	Ambient
Diluent	mixture of 0.1 N sodium hydroxide solution and methanol (75:25). e
Sample:	250 ppm of Lansoprazole



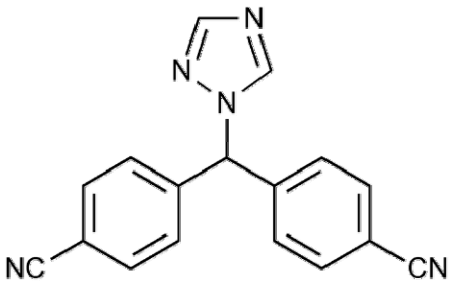
Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution
1	Lansoprazole N-oxide	21.3	1.0	0.8	
2	Lansoprazole	26.6	1.0	1.0	
3	Lansoprazole RS A	28.9	1.1	1.1	8.0
4	Lansoprazole RS B	32.7	1.1	1.2	

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Letrozole

USP Method Letrozole RS
USP Method Letrozole Assay



Original Manufacturer: Novartis (patent expire 2011)

Brand Name: Femara

Letrozole is an oral non-steroidal aromatase inhibitor for the treatment of hormonally-responsive breast cancer after surgery.

Estrogens are produced by the conversion of androgens through the activity of the aromatase enzyme. Estrogens then bind to an estrogen receptor, which causes cells to divide.

Letrozole prevents the aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit. The action is specific, and Letrozole does not reduce production of mineralo- or corticosteroids.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Letrozole

USP34 – NF29 S1

USP Columns:

Nucleosil C18 Assay and Related Compounds 4.6 mm x 12.5 cm, 5 µm

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm	(1.51455.0001)

Recommended Solvents and Reagents:

Acetonitrile	gradient grade for liquid chromatography LiChrosolv®	(1.00030)
Water	Water for chromatography LiChrosolv®	(1.15333)
	or freshly purified water from Milli-Q water purification system	

USP Standards

Letrozole (200 mg)	USP Product Number:	1356971
Letrozole Related Compound A (25 mg)	USP Product Number:	1356982

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Letrozole Assay

Solution A: Water
Solution B: Acetonitrile

Mobile phase
See the gradient table:

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Diluent: Acetonitrile and water (3:7)

Standard solution
10 µg/mL of USP Letrozole RS in Diluent.
[Note—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.] USP34

Sample solution
10 µg/mL of Letrozole in Diluent.
[Note—Dissolve Letrozole in acetonitrile, then dilute with water.] USP34

Chromatographic system (*See Chromatography 621, System Suitability.*)
Detector: UV 230 nm
Column: 4.6-mm × 12.5-cm; 5-µm packing L1
Injection size: 20 µL
Flow rate: 1 mL/min

System suitability *(Sample: Standard solution)*
Suitability requirements
Tailing factor: 0.8–1.5
Relative standard deviation: NMT 2.0%

Analysis: *(Samples: Standard solution and Sample solution)*

Calculate the percentage of $C_{17}H_{11}N_5$ in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution
r_S = peak response from the Standard solution
C_S = concentration of USP Letrozole RS in the Standard solution (mg/mL)
C_U = nominal concentration of Letrozole in the Sample solution (mg/mL)

Acceptance criteria
98.0%–102.0% on the anhydrous basis

USP Method for Letrozole Assay

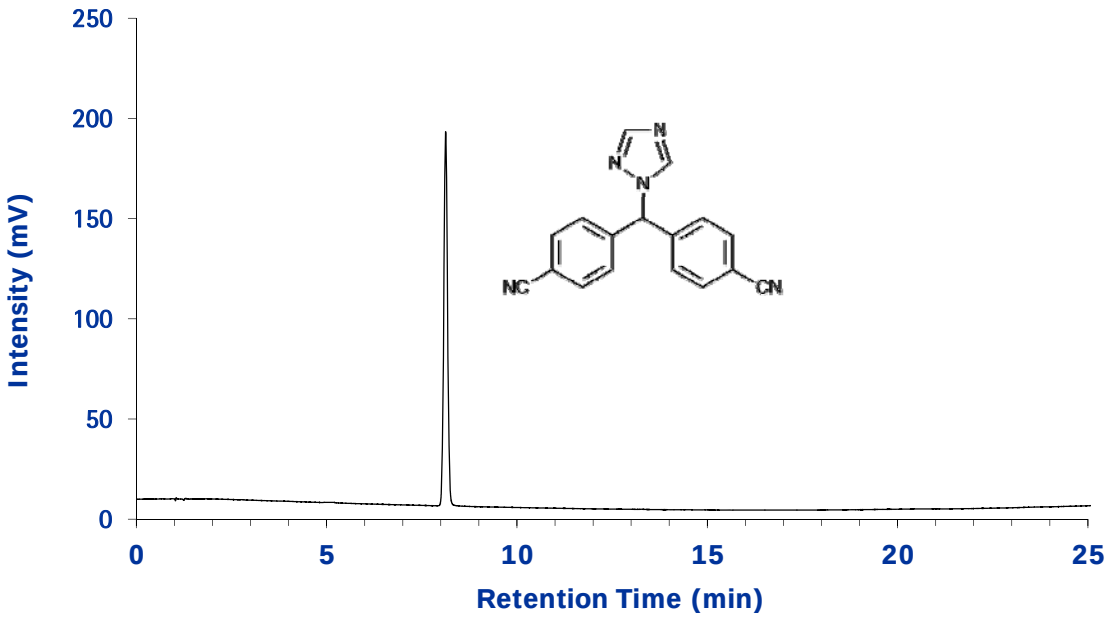
Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@230 nm	
Cell:	Semi-micro cell 2.5µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Water and Solution B: Acetonitrile	
Gradient:	See gradient table	

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Temperature:	Ambient
Diluent:	Acetonitrile and water (3:7)
Sample:	10 µg/mL of USP Letrozole RS (standard solution)
Pressure Drop:	140-96 Bar (2016-1382 psi)



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Letrozole	8.1	1.0	-	1.04

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Letrozole RS

Solution A, Solution B, Mobile phase, Chromatographic system,USP34 and Diluent:
Proceed as directed in the Assay.

System suitability solution
2 µg/mL of USP Letrozole Related Compound A RS and 10 µg/mL of USP Letrozole RS in Diluent.
[Note—Dissolve Letrozole and USP Letrozole Related Compound A RS in acetonitrile, then dilute with water.] USP34

Standard solution
1 µg/mL of USP Letrozole RS in Diluent.
[Note—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.] USP34

Sample solution
Transfer 25 mg of Letrozole to a 250-mL volumetric flask. Dissolve in 75 mL of acetonitrile, and dilute with water to volume. USP34

System suitability *(Samples: System suitability solution and Standard solution (USP34))*
Suitability requirements
Resolution: NLT 2.0
USP34 between Letrozole related compound A and Letrozole (System suitability solution)
Relative standard deviation: NMT 10.0%, Standard solution

Analysis
(Samples: Standard solution and Sample solution)

Calculate the percentage of each impurity in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the Sample solution
r_S = peak response of letrozole from the Standard solution
C_S = concentration of USP Letrozole RS in the Standard solution (mg/mL)
C_U = concentration of Letrozole in the Sample solution (mg/mL)

USP Method for Letrozole RS

Acceptance criteria

Individual impurities: See Impurity Table 1.
Total unspecified impurities: NMT 0.3%
[Note—Disregard any impurity peaks less than 0.05%.] USP34

Table 1.

Compound	Relative Retention Time (RRT)	Limit (%)
Letrozole related compound A[1]	0.67	0.3
Letrozole	1.0	-
4,4',4''-Methanetriyltribenzonitrile	2.4	0.1
Any other individual impurity	-	0.1

1 = 4,4¢-(1H-1,3,4-triazol-1-ylmethylene)dibenzonitrile.

USP Method for Letrozole RS

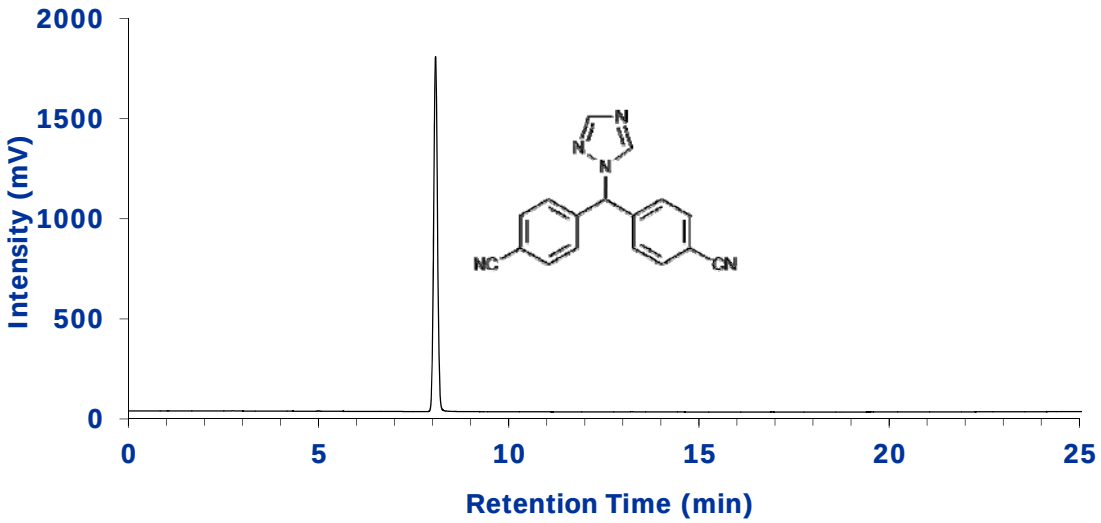
Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@230 nm	
Cell:	Semi-micro cell 2.5µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Water and Solution B: Acetonitrile	
Gradient:	See gradient table	

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Temperature:	Ambient
Diluent:	Acetonitrile and water (3:7)
Sample:	100 ppm (0.1 mg/mL) of USP Letrozole RS (sample solution)
Pressure Drop:	140-96 Bar (2016-1382 psi)



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Letrozole	8.1	1.0	-	1.05

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Letrozole RS – SST solution

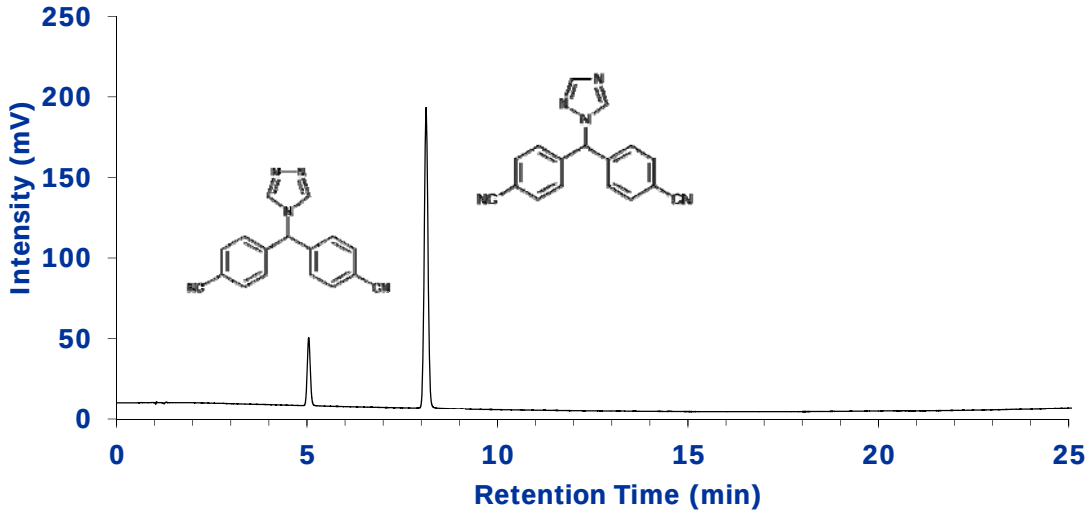
Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@230 nm	
Cell:	Semi-micro cell 2.5µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Water and Solution B: Acetonitrile	
Gradient:	See gradient table	

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Temperature:	Ambient
Diluent:	Acetonitrile and water (3:7)
Sample:	2 µg/mL of USP Letrozole Related Compound A RS and 10 µg/mL of USP Letrozole RS
Pressure Drop:	140-96 Bar (2016-1382 psi)



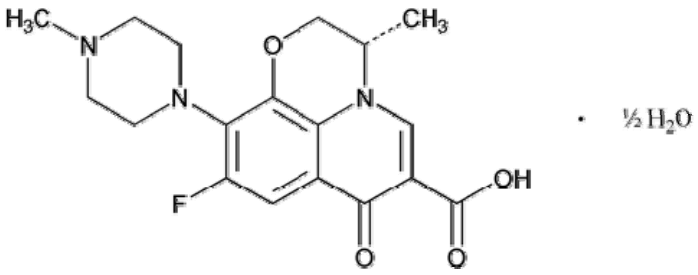
Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Letrozole RS A	5.0	0.6	-	1.07
2	Letrozole	8.1	1.0	18.7	1.04

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

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.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

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

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

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.

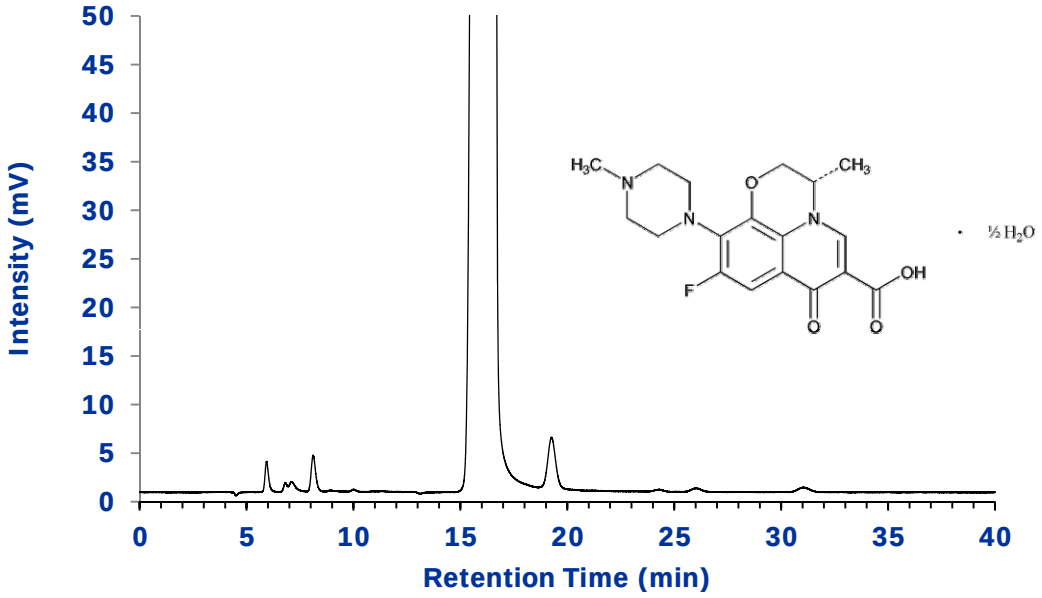
Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

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)	



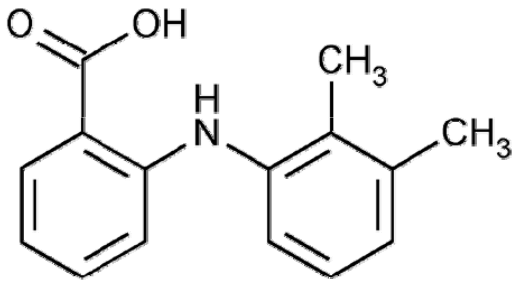
Chromatographic Data

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

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Mefenamic Acid

USP Method Mefenamic Acid RS
USP Method Mefenamic Acid Assay



Original Manufacturer: Shionogi Inc (Patent expired)

Brand Name: Mefalth, Mefalth T, Ponstel, Ponstan, Ponstal, Parkemed, Mafepain, Mefamed, Mephadolor, Meftal, Dyfenamic, Potarlon, Dolfenal, Meyerdonal, Alfoxan, Fenagesic, Spiralgin.

Mefenamic acid is a non-steroidal anti-inflammatory drug used to treat pain, including menstrual pain. Mefenamic acid decreases inflammation (swelling) and uterine contractions .

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Mefenamic Acid

USP34 – NF29 S1

USP Columns:

ZORBAX ODS Assay and Chromatographic purity

Equivalent Column:

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

Recommended Solvents and Reagents:

Acetonitrile isocratic grade for liquid chromatography LiChrosolv® (1.14291)

Tetrahydrofuran for liquid chromatography LiChrosolv® (1.08101)

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

Ammonium phosphate (mono basic) Use ACS Reagent grade

Ammonium Hydroxide Use ACS Reagent grade

USP Standards

Mefenamic Acid (200 mg) USP Product Number: 1379605

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Mefenamic Acid

Buffer solution
Prepare a 50 mM solution of monobasic ammonium phosphate, and adjust with 3 M ammonium hydroxide to a pH of 5.0.

Mobile phase
Prepare a filtered and degassed mixture of acetonitrile, Buffer solution, and tetrahydrofuran (23:20:7). Make adjustments if necessary (*see System Suitability under Chromatography 621*).

Standard preparation
Dissolve an accurately weighed quantity of USP Mefenamic Acid RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation
Transfer about 100 mg of Mefenamic Acid, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

Chromatographic system (*see Chromatography 621*)
The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure:

Column efficiency is not less than 8200 theoretical plates;
Tailing factor for the analyte peak is not more than 1.6;
Relative standard deviation for replicate injections is not more than 1.0%.

Procedure:
Separately inject equal volumes (about 10 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 $C_{15}H_{15}NO_2$ in the portion of Mefenamic Acid taken by the formula:

$500C(r_U/r_S)$ C = concentration in mg/mL, of USP Mefenamic Acid RS in the Standard preparation
r_U and r_S are the Mefenamic acid peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method for Mefenamic Acid RS

Chromatographic purity
Buffer solution, Mobile phase, and Chromatographic system—Proceed as directed in the Assay.

Standard solution
Dissolve an accurately weighed quantity of USP Mefenamic Acid RS in Mobile phase to obtain a solution having a known concentration of about 10 µg per mL.

Test solution
Transfer about 100 mg of Mefenamic Acid, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

Procedure
Separately inject equal volumes (about 10 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 of each impurity in the portion of Mefenamic Acid taken by the formula:

$100(C_S/C_U)(r_i/r_S)$

C_S = conc. in µg/mL, of USP Mefenamic Acid RS in Standard solution
C_U = conc. in µg/mL, of Mefenamic Acid in the Test solution
ri = the peak response for each impurity obtained from the Test solution
rS =the peak response for Mefenamic acid from Standard solution

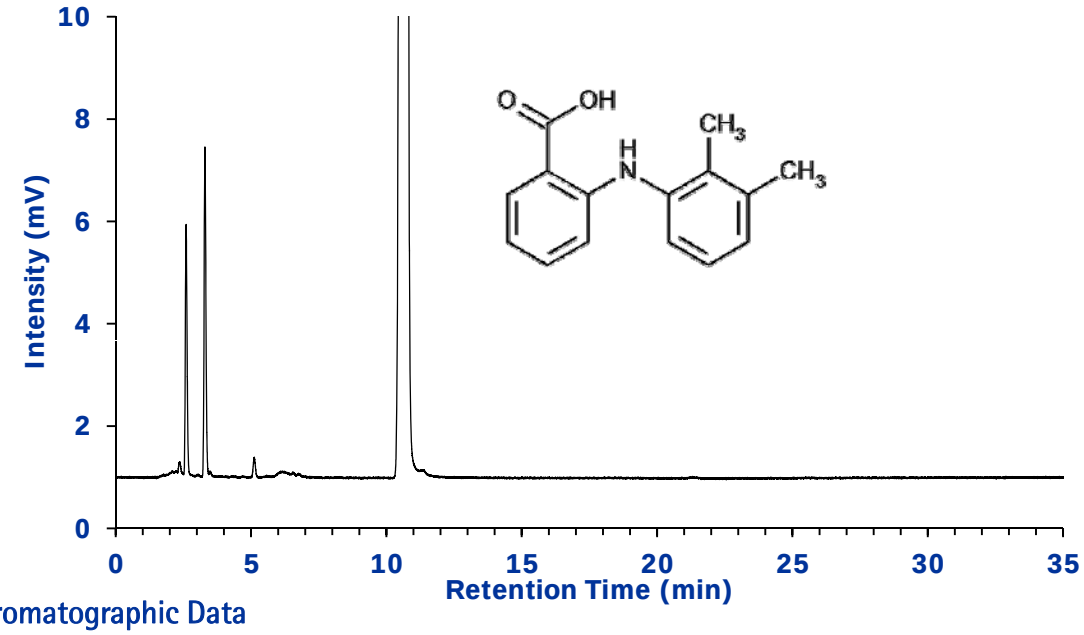
Not more than (NMT) 0.1% of any individual impurity is found; and NMT 0.5% of total impurities is found.

USP Method for Mefenamic Acid RS

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence, UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 50 mM of monobasic ammonium phosphate, adjusted with 3 M ammonium hydroxide to a pH of 5.0. Mix tetrahydrofuran, buffer and acetonitrile (14:40:46)	
Temperature:	25°C	
Diluent	Mobile phase	
Sample:	5 ppm of Impurity C and D, 0.1 ppm of Impurity A and 100 ppm Mefenamic Acid	
Pressure Drop:	140 Bar (2030 psi)	



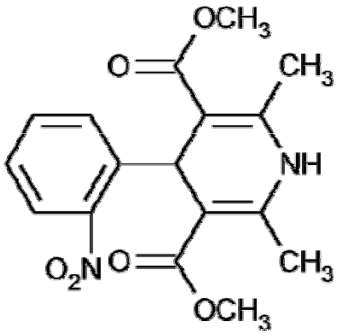
Chromatographic Data

No	Compound	Time (min)	Relative Retention Time (RRT)	Asymmetry (T_{USP})	Plates (N)
1	Impurity C	2.6	0.24	1.2	7697
2	Impurity D	3.3	0.31	1.2	11439
3	Impurity A	5.1	0.48	1.1	19510
4	**Mefenamic acid**	**10.7**	**1.00**	**0.9**	**18758**

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Nifedipine

USP Method Nifedipine RS
USP Method Nifedipine Assay



Original Manufacturer: Bayer (patent expired)

Brand Name: Adalat, Nifediac, Cordipin, Nifedical, and Procardia

Nifedipine is a dihydropyridine calcium channel blocker. Its main uses are as an antianginal (especially in Prinzmetal's angina) and antihypertensive, although a large number of other indications have recently been found for this agent, such as Raynaud's phenomenon, premature labor, and painful spasms of the esophagus in cancer and tetanus patients. It is also commonly used for the small subset of pulmonary hypertension patients whose symptoms respond to calcium channel blockers.

The approved uses for Nifedipine are the long-term treatment of hypertension (high blood pressure) and angina pectoris.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Nifedipine

USP34 – NF29 S1

USP Columns:

C-18-IP Ultrasphere. Assay and Related Compounds 5-µm, Beckman Instruments.
Alternative column Luna C18(2), Phenomenex

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm (1.50252.0001)

Recommended Solvents and Reagents:

Acetonitrile	isocratic grade for liquid chromatography LiChrosolv®	(1.14291)
Methanol	for liquid chromatography LiChrosolv®	(1.06018)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	(1.15333)

USP Standards

Nifedipine (125 mg)	USP Product Number:	1463508
Nifedipine Nitrophenylpyridine Analog (25 mg)	USP Product Number:	1463600
Nifedipine Nitrosophenylpyridine Analog (25 mg)	USP Product Number:	1463701

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Nifedipine Assay

Assay

[note—Protect the Standard preparation and the Assay preparation from actinic light. Conduct the Assay promptly after preparation of the Standard preparation and the Assay preparation.]

Mobile phase

Prepare a suitable mixture of water, acetonitrile, and methanol (50:25:25), and degas.
Make adjustments if necessary (*see System Suitability under Chromatography 621*).

Standard preparation

Dissolve an accurately weighed quantity of USP Nifedipine RS in methanol (about 1 mg per mL), and quantitatively dilute with Mobile phase to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation

Transfer about 25 mg of Nifedipine, accurately weighed, to a 250-mL volumetric flask. Dissolve in 25 mL of methanol, dilute with Mobile phase to volume, and mix to obtain a solution having a concentration of about 0.1 mg per mL.

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure:

Column efficiency is not less than 4000 theoretical plates;
Tailing factor is not more than 1.5;
Relative standard deviation for replicate injections is not more than 1.0%.

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 responses for the major peaks.
Calculate the quantity, in mg, of $C_{17}H_{18}N_2O_6$ in the portion of Nifedipine taken by the formula:

$$250C(r_U/r_S)$$

C = concentration in mg/mL, of Nifedipine RS in the Standard preparation, and where r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method for Nifedipine RS

Related compounds

[note—Protect the Standard Nifedipine solution and the Test preparation from actinic light. Conduct this test promptly after preparation of the Standard Nifedipine solution and the Test solution.]

Mobile phase: *(Prepare as directed in the Assay)*

Standard Nifedipine solution

Dissolve an accurately weighed quantity of USP Nifedipine RS in methanol (about 1 mg per mL), and dilute quantitatively with Mobile phase to obtain a solution having a known concentration of about 0.3 mg per mL.

Reference solution 1:

Dissolve an accurately weighed quantity of USP Nifedipine Nitrophenylpyridine Analog RS in methanol (about 1 mg per mL), and dilute quantitatively with Mobile phase to obtain a solution having a known concentration of about 0.6 µg per mL.

Reference solution 2

Dissolve an accurately weighed quantity of USP Nifedipine Nitrosophenylpyridine Analog RS in methanol (about 1 mg per mL), and dilute quantitatively with Mobile phase to obtain a solution having a known concentration of about 0.6 µg per mL.

Standard solution

Transfer 5.0 mL of each of the two Reference solutions to a container, add 5.0 mL Mobile phase, and mix.

Test solution

Prepare as directed for the Assay preparation in the Assay.

System suitability solution

Mix equal volumes of the Standard Nifedipine solution and of each of the two Reference solutions.

Chromatographic system

Prepare as directed in the Assay. Chromatograph the System suitability preparation, and record the peak responses as directed for Procedure: Resolution, R, between the nitrophenylpyridine analog and nitrosophenylpyridine analog peaks is not less than (NLT) 1.5; the resolution, R, between the nitrosophenylpyridine analog and Nifedipine peaks is NLT 1.0; and the relative standard deviation of the response for each analog in replicate injections is not more than 10%. The relative retention times are about 0.8 for the nitrophenylpyridine analog, about 0.9 for the nitrosophenylpyridine analog, and 1.0 for Nifedipine.

Procedure

Separately inject equal volumes (about 25 µL) of the Standard and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of each related compound in the portion of Nifedipine taken by the formula:

$250C(r_U/r_S)$ C= conc. in mg/mL, of the appropriate USP Nifedipine Analog RS, in the Standard solution; r_U and r_S = peak responses for the corresponding related compound obtained from the Test solution and the Standard solution, respectively.

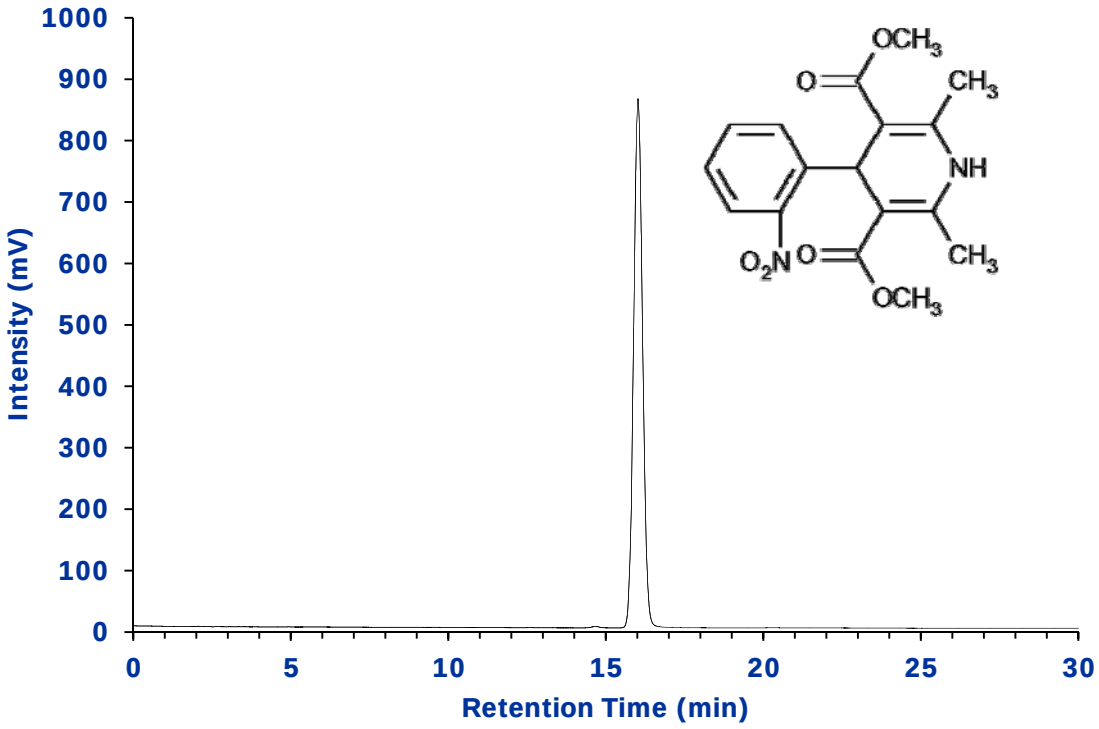
NMT 0.2% of each of dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate and dimethyl- 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate, corresponding to Nifedipine Nitrophenylpyridine Analog and Nifedipine Nitrosophenylpyridine Analog, respectively, is found.

USP Method for Nifedipine

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 250x4.0 mm	1.50252.0001
Injection:	25 µL	
Detection:	Shimadzu Prominence, UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Acetonitrile, Methanol and Water in a mixture (25:25:50)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	100 ppm (0.1 mg/mL) of Nifedipine	
Pressure Drop:	208 Bar (3016 psi)	



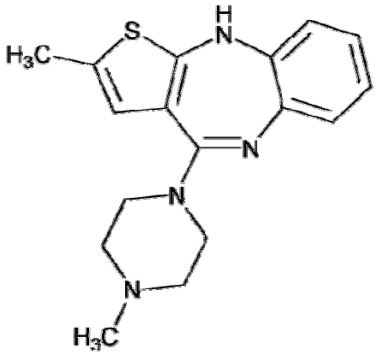
Chromatographic Data

No	Compound	Time (min)	Relative Retention Time (RRT)	Asymmetry (T_{USP})	Plates (N)
1	Nifedipine	16.0	1.00	1.1	15035

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Olanzapine

USP Method Olanzapine Assay



Original Manufacturer:	Eli Lilly (patent expired October 23, 2011)
Brand Name:	Zyprexa, Zalasta, Zolafren, Olzapin, Oferta, Zypadhera
Combination Drug:	Symbyax (Olanzapine and Fluoxetine)

Olanzapine is an atypical antipsychotic, approved for the treatment of schizophrenia and bipolar disorder.

Sales of Zyprexa in 2010 was $5.7 Billion in total.

US federal health officials approved the first generic versions of the blockbuster drug Zyprexa, October 25, 2011. The new low-cost versions of the drug will be marketed by Indian generic drug maker Dr. Reddy's Laboratories and by Teva Pharmaceutical Industries, based in Israel.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Olanzapine

USP34 – NF29 S1

USP Columns:

ZORBAX RX-C8: Assay 4.6 mm x 15 cm, Related Compounds 4.6 mm x 25 cm, both 5 µm.

Equivalent Column:

Purospher®STAR RP-8 endcapped (5 µm) 250x4.6 mm (1.51454.0001)

Recommended Solvents and Reagents:

Acetonitrile isocratic grade for liquid chromatography LiChrosolv® (1.14291)

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

Sodium dodecyl sulfate for ion pair chromatography LiChropur® (1.18309)
Phosphoric Acid Use ACS reagent grade
Sodium di-hydrogen phosphate Use ACS reagent grade

USP Standards

Olanzapine (200 mg)	USP Product Number:1478301
Olanzapine Related Compound A (25 mg)	USP Product Number:1478312
Olanzapine Related Compound B (25 mg)	USP Product Number: 1478323

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method Olanzapine Assay

Mobile phase
Dissolve 6.9 g of monobasic sodium phosphate in 1 L water, adjust the pH to 2.5 with phosphoric acid, then add 12 g of sodium dodecyl sulfate per L, and mix. Prepare a filtered and degassed mixture of this buffer and acetonitrile (53:47). Make adjustments if necessary (*see System Suitability under Chromatography 621*).

System suitability solution
Dissolve suitable quantities of USP Olanzapine RS and USP Olanzapine Related Compound A RS in Mobile phase to obtain a final solution having a concentration of about 0.1 mg of Olanzapine and 0.01 mg per mL of Olanzapine related compound A, respectively.

Standard preparation
Dissolve an accurately weighed quantity of USP Olanzapine RS in Mobile phase, and dilute quantitatively with Mobile phase to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation
Dissolve an accurately weighed quantity of Olanzapine in Mobile phase, to obtain a solution having a nominal concentration of about 0.1 mg per mL.

Chromatographic system (*see Chromatography 621*)
The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm × 15-cm column that contains 5-µm L7 packing. The flow rate is about 1.5 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure:

- the resolution, R, between Olanzapine related compound A and Olanzapine is not less than 2.0
- the tailing factor for Olanzapine peak is between 0.8 and 1.5;
- the relative standard deviation for replicate injections for the Olanzapine peak is not more than 1.0%.

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 percentage of $C_{17}H_{20}N_4S$ in the portion of Olanzapine taken by the formula:

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

in which C_S and C_U are the concentrations, in mg per mL, of Olanzapine in the Standard preparation and the Assay preparation, respectively; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method Olanzapine RS

Buffer solution
Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water, add 5 mL of phosphoric acid, and adjust the pH to 2.5 with a sodium hydroxide solution.

Solution A: Mix the Buffer solution and acetonitrile (52:48). Filter and degas.

Solution B: Mix acetonitrile and the Buffer solution (70:30). Filter and degas.

Mobile phase
Use variable mixtures of Solution A and Solution B. Make adjustments if necessary *(see System Suitability under Chromatography 621)*.

Diluent
Dissolve about 55 mg of edetate disodium in 1500 mL of Buffer solution. Prepare a mixture of the resulting solution and acetonitrile (3:2).

System suitability solution
Dissolve suitable quantities of USP Olanzapine RS, USP Olanzapine Related Compound A RS, and USP Olanzapine Related Compound B RS in Diluent to obtain a solution having a concentration of about 20 µg per mL of Olanzapine and 2 µg per mL each of Olanzapine related compound A and Olanzapine related compound B, respectively.

Standard solution
Dissolve an accurately weighed quantity of USP Olanzapine RS in Diluent to obtain a solution having a known concentration of about 0.002 mg per mL.

Test solution
Transfer about 10 mg of Olanzapine, accurately weighed, to a 25-mL volumetric flask. Dissolve in and dilute with Diluent to volume, and mix.

Chromatographic system (*see Chromatography 621*)
The liquid chromatograph is equipped with a 220-nm detector, and a 4.6-mm × 25-cm column that contains 5-µm L7 packing, is maintained at a constant temperature of about 35, and is programmed to provide a Mobile phase consisting of variable mixtures of Solution A and Solution B. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (min)	Solution A (%)	Solution B (%)	Elution
0-10	100	0	Isocratic
10-20	100→0	0→100	Linear gradient
20-25	0	100	Isocratic
25-27	0→100	100→0	Linear gradient
27-35	100	0	Isocratic

USP Method Olanzapine RS

Chromatograph the System suitability solution, and record the peak responses as directed for Procedure. Identify the peaks using the approximate RRT values given in Table 1;

•the resolution, R, between Olanzapine related compound A and Olanzapine is not less than 3.0;
•the tailing factor for the Olanzapine peak is not more than 1.5;
•the relative standard deviation for four replicate injections for the Olanzapine peak is NMT 2.0%.

Peak Identification	Rel. Ret. Time (RRT)	Rel. Resp. Factor (RRF)	Limit (%)
Olanzapine RS B	0.3	2.3	0.1
Olanzapine RS A	0.8	1.0	0.1
Olanzapine	**1.0**	**-**	**-**
Any individual unspecific impurity	-	-	0.1
Total	-	-	0.4
Olanzapine RS A = 5-Methyl-2-((2-nitrophenyl)amino)-3-thiophenecarbonitrile			
Olanzapine RS B = 2-Methyl-10H-thieno-[2,3-b][1,5] benzodiazepin-4[5H]-one			

Procedure

Separately inject equal volumes (about 20 µL) of the Standard solution and the Test solution, maintained at a temperature of about 5 degrees Celsius, into the chromatograph, record the peaks, and measure the responses for the peaks. Calculate the percentage of each impurity in the portion of Olanzapine taken by the formula:

$$100(1/F)(C_S / C_T)(r_i / r_S)$$

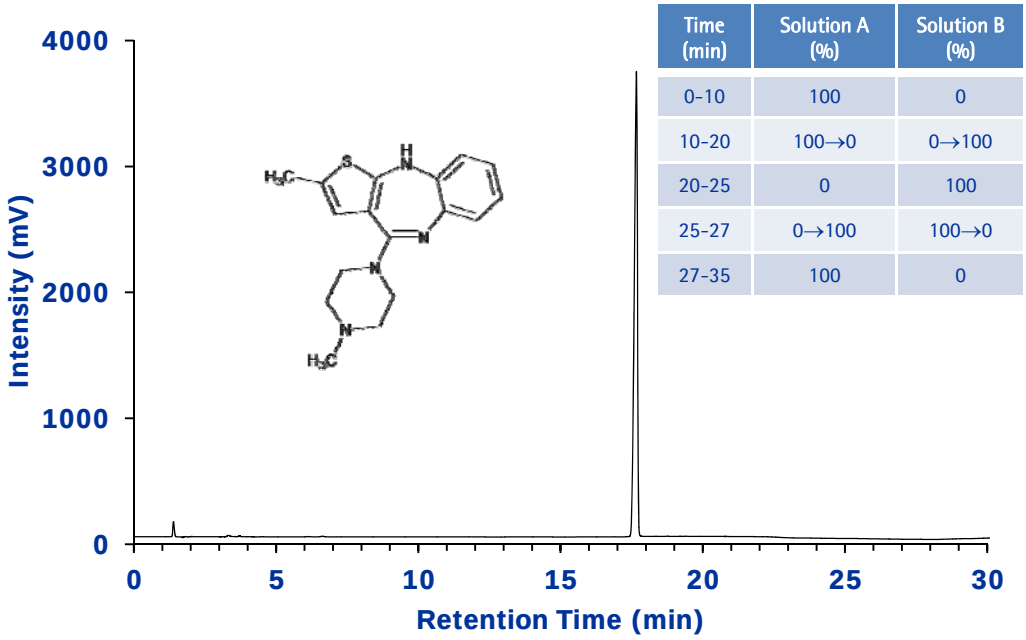
in which F is the relative response factor for each impurity from Table 1; C_S is the concentration, in mg per mL, of USP Olanzapine RS in the Standard solution; C_T is the concentration, in mg per mL, of Olanzapine in the Test solution; r_i is the response for each impurity in the Test solution; and r_S is the response of the Olanzapine peak in the Standard solution. The impurity limits are given in Table 1.

USP Method for Olanzapine RS

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-8 endcapped (5 µm) 250x4.6 mm	1.51454.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@220 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase (v/v):	Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water, add 5 mL of phosphoric acid, and adjust the pH to 2.5 with a sodium hydroxide solution. Solution A: Buffer solution and acetonitrile (52:48) Solution B: acetonitrile and the Buffer solution (70:30)	
Gradient:	See Table:	
Temperature:	30° Celsius	
Diluent	55 mg of disodium EDTA in 1500 ml buffer. Mix resulting solution & acetonitrile (3:2)	
Sample:	Test solution - 400 ppm (0.4 mg/mL) of Olanzapine	
Pressure Drop:	146 Bar (2117 psi)	



Time (min)	Solution A (%)	Solution B (%)	Elution
0-10	100	0	Isocratic
10-20	100→0	0→100	Linear gradient
20-25	0	100	Isocratic
25-27	0→100	100→0	Linear gradient
27-35	100	0	Isocratic

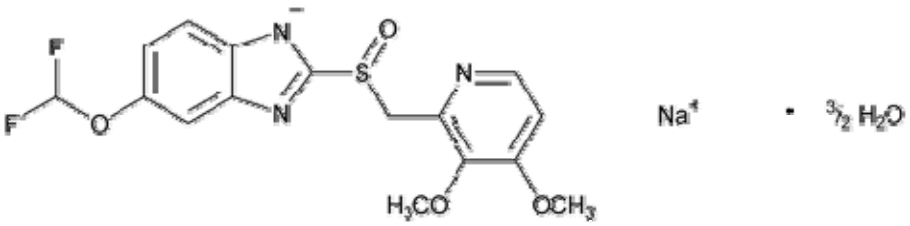
Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Olanzapine	17.7	0.74	1.0	

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Pantoprazole Sodium

USP Method Pantoprazole Sodium RS
USP Method Pantoprazole Sodium Assay



Original Manufacturer:	Altana (Nycomed) and licensed in the USA to Wyeth (patent expiry 2009)
Brand Names:	Protonix, Somac, Pantoloc, Protium, Pantecta, Pantoheal, Fenix, Tecta, Protium, Inipomp, Eupantol, Pantozol, Pantodac, Perizole, Pansped, Zurcazol, Protonex, Pantup, Pantomed, TopZole, UXL-D, Pantid
Combination Drugs:	Pantazone (Domperidone and Pantoprazole) Pantop-D (Domperidone and Pantoprazole) Rantop-D (Domperidone and Pantoprazole)

Pantoprazole is a proton pump inhibitor drug that inhibits gastric acid secretion.

In 2007, Teva Pharmaceutical released an AB-rated generic alternative to Protonix. This was followed by generic equivalents from Sun Pharma and Kudco Pharma. Wyeth sued all three for patent infringement and launched its own generic version of Protonix with Nycomed.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Pantoprazole Sodium

USP34 – NF29 S1

USP Columns:

Luna C18 Assay and Related Compounds 3.9 mm x 15 cm, 4 µm.

Hypersil-ODS Related compounds Related Compounds Test 2. 4 mm x 12.5 cm, 5 µm.

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm	(1.51455.0001)
Purospher®STAR RP-18 endcapped (5 µm) 125x4.0 mm	(1.50036.0001)

Recommended Solvents and Reagents:

Acetonitrile	gradient grade for liquid chromatography LiChrosolv®	(1.00030)
Methanol	gradient grade for liquid chromatography LiChrosolv®	(1.06007)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	**(1.15333)**

Ammonium phosphate (mono basic)	Use ACS Reagent grade
Phosphoric Acid	Use ACS reagent grade
Ammonium Hydroxide	Use ACS Reagent grade
Potassium Phosphate, Dibasic	Use ACS Reagent grade

USP Standards

Pantoprazole Sodium (250 mg)	USP Product Number:	1494895
Pantoprazole Related Compound A (25 mg)	USP Product Number:	1494909
Pantoprazole Related Compound B (20 mg)	USP Product Number:	1494910
Pantoprazole Related Compound C (20 mg)	USP Product Number:	1494920
Pantoprazole Related Compounds D and F Mixture (20 mg)		1494931
Pantoprazole Related Compound E (20 mg)	USP Product Number:	1494942

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Pantoprazole Sodium Assay

[note—Protect all solutions from light, and use amber autosampler vials and low-actinic glassware.]

Ammonium phosphate buffer

Dissolve 1.32 g of dibasic ammonium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.5.

Acetonitrile–methanol mixture Prepare a mixture of acetonitrile and methanol (7:3).

Solution A

Use a filtered and degassed mixture of Ammonium phosphate buffer and Acetonitrile–methanol mix (85:15).

Solution B

Use Acetonitrile–methanol mixture.

Diluent

Transfer 25 mL of ammonium hydroxide to a suitable container, and dilute with water to 500 mL.

Mobile phase

Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary (*see System Suitability under Chromatography 621*).

System suitability preparation

Dissolve suitable amounts of USP Pantoprazole Sodium RS, USP Pantoprazole Related Compound A RS, and USP Pantoprazole Related Compound B RS in a mixture of acetonitrile and water (1:1) to obtain a solution having about 0.5 mg of each component per mL. Transfer 1 mL of this solution to a 100-mL volumetric flask, and dilute with Diluent to volume.

Standard preparation

Transfer about 20 mg of USP Pantoprazole Sodium RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), and dilute with Diluent to volume. Further dilute with Diluent quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.06 mg per mL.

Assay preparation

Transfer about 20 mg of Pantoprazole Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), and dilute with Diluent to volume. Further dilute with Diluent quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.06 mg per mL.

USP Method for Pantoprazole Sodium Assay

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 285-nm detector and 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 30, and the autosampler temperature is maintained at 4. The chromatograph is programmed as follows:

Time (min)	Solution A (%)	Solution B (%)	Elution
0-10	86	14	Isocratic
10-35	86→42	14→58	Linear gradient
35-36	42→86	58→14	Linear gradient
36-46	86	14	re-equilibration

Chromatograph the System suitability preparation, and record the peak responses as directed for Procedure. Identify the components based on their relative retention times (Table 1): the resolution, R, between the Pantoprazole related compound A and Pantoprazole peaks is not less than 10.0. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: 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 percentage of $C_{16}H_{14}F_2N_3NaO_4S$ in the portion of Pantoprazole Sodium taken by the formula:

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

in which C_S and C_U are the concentrations, in mg/mL, of Pantoprazole sodium in the Standard preparation and the Assay preparation, respectively; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method for Pantoprazole Sodium RS

[On the basis of the synthetic route, perform either Test 1 or Test 2.
Test 2 is recommended when impurities C, D, E, and F are potential related compounds.]

Test 1 *[note—Protect all solutions from light, and use amber autosampler vials and low-actinic glassware.]*
Diluent, Mobile phase, System suitability preparation, and Chromatographic system—Proceed as directed in the Assay.

Standard solution
Transfer about 20 mg of USP Pantoprazole Sodium RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), and dilute with Diluent to volume. Further dilute with Diluent quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.0004 mg/mL.

Test solution
Transfer about 20 mg of Pantoprazole Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), dilute with Diluent to volume, and mix.

Chromatographic system (*see Chromatography 621*)
Prepare as directed in the Assay. Chromatograph the System suitability preparation, and record the peak responses as directed for Procedure. Identify the components on the basis of their relative retention times (Table 1): the resolution, R, between the Pantoprazole related compound A and Pantoprazole peaks is not less than 10.0.

Procedure
Separately inject equal volumes (about 20 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Pantoprazole Sodium taken by the formula:

$100(C_S / C_T)(r_i/r_S)$

C_S and C_T = conc. in mg/mL, of Pantoprazole sodium in the Standard solution and the Test solution, respectively;
r_i = peak response of each impurity obtained from the Test solution;
r_S= Pantoprazole peak response obtained from the Standard solution.

The reporting level for impurities is 0.05%.

Table 1.

Compound	Relative Retention Time (RRT)	Limit (%)
Pantoprazole related compound A[1)]	0.52	0.20
Pantoprazole sodium	1.0	-
Pantoprazole related compound B[2)]	1.7	0.15
Any other individual impurity	1.5	0.1
Total impurities	-	0.5

[1)] 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfonyl]-1H-benzimidazole.

[2)] 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]thio]-1H-benzimidazole.

USP Method for Pantoprazole Sodium RS – Test 2

Diluent
Prepare a mixture of acetonitrile and 0.001 N sodium hydroxide solution (50:50).

Standard solution
Dissolve an accurately weighed quantity of USP Pantoprazole Sodium RS in Diluent, and dilute quantitatively to obtain a solution having a known concentration of about 0.03 mg/mL.

Test solution
Prepare a solution of Pantoprazole Sodium in Diluent with known concentration of about 0.46 mg/mL.

System suitability solution
Dissolve suitable amounts of USP Pantoprazole Sodium RS, USP Pantoprazole Related Compound A RS, USP Pantoprazole Related Compound B RS, USP Pantoprazole Related Compound C RS, USP Pantoprazole Related Compound D and F Mixture RS, and USP Pantoprazole Related Compound E RS in Diluent to obtain a solution containing about 0.46 mg of Pantoprazole sodium per mL and about 1.3 µg each of related compounds A, B, C, and E per mL, and about 1.3 µg of the D and F mixture per mL.

Solution A
Prepare a solution of dibasic potassium phosphate (1.74 g/L) adjusted with a solution of phosphoric acid (330 g/L) to a pH of 7.00 ± 0.05.

Solution B
Use acetonitrile.

Mobile phase
Use variable mixtures of Solution A and Solution B as directed below for Chromatographic system. Make adjustments as necessary (*see System Suitability under Chromatography 621*).

Chromatographic system (*see Chromatography 621*)
The liquid chromatograph is equipped with either a programmable variable wavelength detector or two separate detectors capable of monitoring at 290 nm and at 305 nm, and a 4-mm × 12.5-cm column that contains 5-µm packing L1. The column temperature is maintained at 40 degrees Celsius. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (min)	Solution A (%)	Solution B (%)	Elution
0-40	80→20	20→80	Linear gradient
40-45	20→80	80→20	Linear gradient
45-55	80	20	re-equilibration

USP Method for Pantoprazole Sodium RS – Test 2

Chromatograph the System suitability solution, and record the peak responses at 290 nm as directed for Procedure. Identify the components based on relative retention times (Table 2): the resolution, R, between Pantoprazole related compound E and Pantoprazole related compounds D and F is not less than 1.5. Chromatograph the Standard solution at 290 nm, and record the peak responses as directed for Procedure: the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure

Separately inject equal volumes (about 20 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms at 290 nm and 305 nm, and measure the responses for the major peaks. [note—Pantoprazole related compound C is monitored using a wavelength of 305 nm, and all other compounds are monitored at 290 nm.] Calculate the percentage of each impurity in the portion of Pantoprazole Sodium taken by the formula:

$100(1/F)(C_S/ C_U)(r_U/r_S)$

C_S = conc. in mg/mL, of Pantoprazole sodium in the Standard solution
C_U = conc. in mg/mL of Pantoprazole Sodium in the Test solution
F = the response factor of an individual Pantoprazole related compound relative to the response of Pantoprazole sodium (Table 2)
r_i = the peak response of each impurity obtained from the Test solution
r_S =the Pantoprazole peak response obtained from Standard solution.

Impurity Name	Rel. Ret. Time (RRT)	Rel. Resp. Factor (RRF)	Limit (%)
Pantoprazole related compound A	0.9	1.0	0.2
Pantoprazole related compound B	1.5	1.0	0.15
Pantoprazole related compound C[1]	0.6	3.3	0.1[2]
Pantoprazole related compound D[3] and F[5]	1.2	1.0	0.2[4]
Pantoprazole related compound E[6]	1.3	1.0	0.1
Any other individual impurity	-	-	0.1
Total impurities	-	-	0.5

[1] 5-(Difluoromethoxy)-1H-benzimidazole-2-thiol.

[2] At 305 nm.

[3] 5-(Difluoromethoxy)-2-[(RS)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1H-benzimidazole.

[4] Impurities D and F are not fully resolved and should be integrated together.

[5] 6-(Difluoromethoxy)-2-[(RS)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1H-benzimidazole.

[6] Mixture of the stereoisomers of 6,6¢-bis(difluoromethoxy)-2,2¢-bis[[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1H,1¢H-5,5¢-bibenzimidazolyl.

The Reporting level for impurities is 0.05%.

HPLC Application note 2029
USP Method Pantoprazole Sodium RS – Test 2

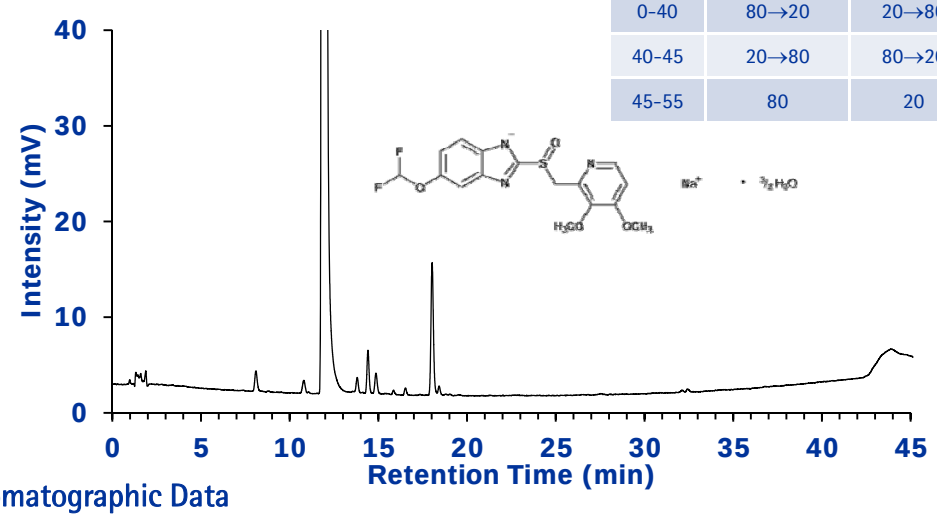
USP Method for Pantoprazole Sodium RS

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence, UV 290 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Solution A: Prepare a solution of dibasic potassium phosphate (1.74 g/L) adjusted with a solution of phosphoric acid (330 g/L) to a pH of 7.00 ± 0.05. Solution B: acetonitrile	
Gradient:	see gradient table	
Temperature:	40°C	
Diluent	1:1 mixture of acetonitrile and 1 mM NaOH	
Sample:	460 ppm of Pantoprazole in Diluent	
Pressure Drop:	101 to 56 Bar (1465 to 812 psi)	

Time (min)	Solution A (%)	Solution B (%)	Elution
0-40	80→20	20→80	Linear gradient
40-45	20→80	80→20	Linear gradient
45-55	80	20	re-equilibration



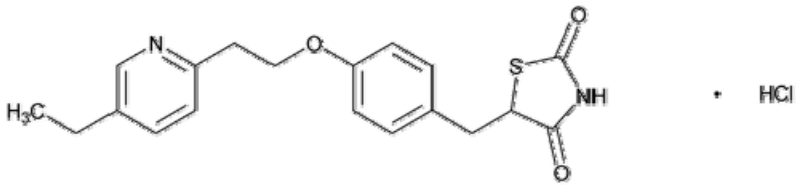
Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Pantoprazole RS C	8.1	0.7	-	1.1
2	Pantoprazole RS A	10.8	0.9	-	1.0
	Pantoprazole Na	11.9	1.0	-	1.1
3	Pantoprazole RS F	13.8	1.2	-	1.1
4	Pantoprazole RS D	14.4	1.2	2.9	1.1
5	Pantoprazole RS E	14.7	1.2	2.0	1.1
6	Pantoprazole RS B	18.0	1.5	-	1.2

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Pioglitazone

USP Method Pioglitazone HCl RS
USP Method Pioglitazone HCl Assay



Original Manufacturer:	Takeda Pharmaceuticals (patent started expire 2011)
Brand Names:	Actos, Glustin , Glizone, Pioz, CND, USV, Zactos
Combination Drugs:	ActoplusMet (Pioglitazone and Metformin) Competact (Pioglitazone and metformin) Duetact (Pioglitazone and Glimepiride)

Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action.

Actos was the tenth-best selling drug in the U.S. in 2008, with sales exceeding $2.4 billion

The first patent for Pioglitazone expired in 2011. However, there is very good reason to believe that other Actos patents (which expire later) will provide protection against generic competition for this medication.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Pioglitazone Hydrochloride

USP34 – NF29 S1

USP Columns:

YMC 18 ODS-A Assay and Organic Impurities 4.6 mm x 15 cm, 5 µm

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm	(1.51455.0001)

Recommended Solvents and Reagents:

Acetonitrile	isocratic grade for liquid chromatography LiChrosolv®	(1.14291)
Methanol	for liquid chromatography LiChrosolv®	(1.06018)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	(1.15333)
Benzophenone		(8.01801)
Ammonium Acetate	Use ACS reagent grade.	
Acetic Acid	Acetic acid (glacial) 100%. Use ACS reagent grade.	

USP Standards

Pioglitazone Hydrochloride (50 mg)	USP Product Number:	1539905

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Pioglitazone Assay

Mobile phase
Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Standard solution
Prepare a 0.5 mg/mL solution of USP Pioglitazone Hydrochloride RS in methanol, and dilute with Mobile phase to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.

System suitability stock solution
0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol

System suitability solution
Dilute System suitability stock solution with Mobile phase to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride and 13 µg/mL of benzophenone.

Sample solution
Prepare a 0.5 mg/mL solution of pioglitazone hydrochloride in methanol, and dilute with Mobile phase to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.

Chromatographic system (*See Chromatography 621, System Suitability.*)
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 2.5°C
Flow rate: 0.7 mL/min
Injection size: 20 µL
[Note—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

System suitability (*Samples: System suitability solution and Standard solution)*
•Approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.

Suitability requirements
•Tailing factor: NMT 1.5 for pioglitazone and benzophenone, System suitability solution
•Resolution: NLT 15 between pioglitazone and benzophenone, System suitability solution
•Relative standard deviation: NMT 2.0% for six replicate injections, Standard solution

Analysis (Samples: Standard solution and Sample solution)
Calculate the percentage of $C_{19}H_{20}N_2O_3S{\cdot}HCl$ in the portion of Pioglitazone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution
r_S = peak response from the Standard solution
C_S = concentration of USP Pioglitazone Hydrochloride RS in the Standard solution (µg/mL)
C_U = concentration of Pioglitazone Hydrochloride in the Sample solution (µg/mL)

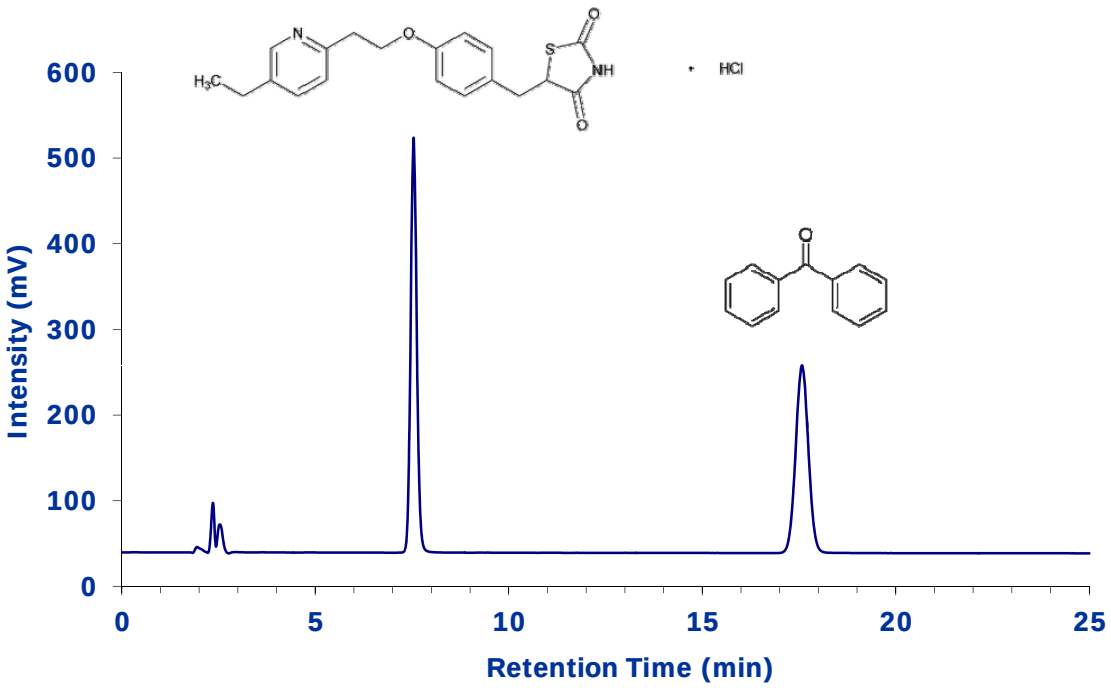
Acceptance criteria:
98.0%–102.0% on the dried basis

USP Method for Pioglitazone HCl Assay

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	VWR-Hitachi LaChrom Elite DAD@269 nm	
Cell:	13 µL	
Flow Rate:	0.7 mL/min	
Mobile Phase (v/v):	Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	50 µg/mL of Pioglitazone HCl and 13 µg/mL of benzophenone (SST solution)	
Pressure Drop:	67 Bar (972 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Pioglitazone HCl	7.6	1.0	0.0	1.09
2	Benzophenone (SST)	17.4	2.3	22.0	1.03

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Analysis protocol for Pioglitazone HCl Assay

USP Method Repeatability

No.	Compound	Response (Arbitrary Area Units)	Relative Standard Deviation (%)	N
1	Pioglitazone HCl	5261746	0.2	6

Replicate injections of standard solution (n=6) were analyzed to determine the USP method repeatability. Sample contained 50 ppm of Pioglitazone HCl in mobile phase.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

No.	Compound	LOD (ppm)	LOQ (ppm)	Curve Equation	Regression Coefficient (R^2)
1	Pioglitazone HCl	0.23	0.71	y = 107244x + 30717	1.000

For Pioglitazone HCl, injections were carried out of at seven different concentrations (25-75 ppm) to determine the linearity of the method.

USP Method for Pioglitazone HCl RS

Mobile phase and System suitability stock solution (*Proceed as directed in the Assay.*)

System suitability solution:
Dilute the System suitability stock solution with Mobile phase to obtain a solution containing 25 µg/mL of Pioglitazone hydrochloride and 6.5 µg/mL of Benzophenone.

Sample solution:
0.2 mg/mL of Pioglitazone hydrochloride dissolved in 20% of the final volume with methanol, then diluted with Mobile phase to final volume

Standard solution
1 µg/mL of Pioglitazone HCl prepared by diluting Sample solution with Mobile phase

Chromatographic system (*See Chromatography 621, System Suitability.*)
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 2.5
Flow rate: 0.7 mL/min
Injection size: 40 µL
Run time: At least four times the retention time of Pioglitazone
[Note—Adjust the flow rate so that the retention time of the Pioglitazone peak is about 7 min.]

System suitability (*Samples: System suitability solution and Standard solution*)
Suitability requirements
•Tailing factor: NMT 1.5 for Pioglitazone and Benzophenone, System suitability solution
•Resolution: NLT 15 between Pioglitazone and Benzophenone, System suitability solution
•Relative standard deviation: NMT 3.0%, Standard solution

Analysis (*Samples: Standard solution and Sample solution*)
Calculate the percentage of each impurity in the portion of Pioglitazone Hydrochloride taken:

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

r_U = = peak response of each individual impurity from the Sample solution
r_S = = peak response of Pioglitazone from the Standard solution
D = = dilution factor used to prepare the Standard solution, 0.005

USP Method for Pioglitazone HCl RS

Acceptance criteria

Individual impurities: See Impurity Table 1.
Total impurities: NMT 0.5%

Table 1.

Compound	Relative Retention Time (RRT)	Limit (%)
Hydroxypioglitazone[a]	0.7	0.2
Pioglitazone	1.0	0.2
Didehydropioglitazone[b]	1.4	0.3
N-Alkylpioglitazone[c]	3.0	0.5
Any other individual impurity	-	0.2

a = (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-5-hydroxythiazolidine-2,4-dione.

B =(Z)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzylidene}thiazolidine-2,4-dione.

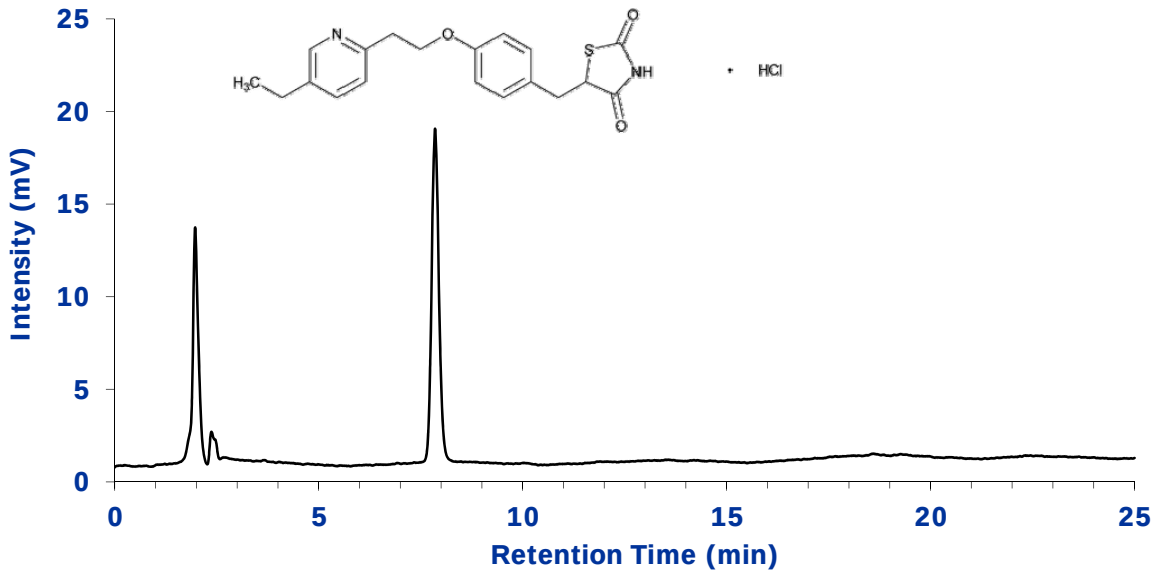
c = (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione.

USP Method for Pioglitazone HCl RS

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	40 µL	
Detection:	VWR-Hitachi LaChrom Elite DAD@269 nm	
Cell:	13 µL	
Flow Rate:	0.7 mL/min	
Mobile Phase (v/v):	Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1 µg/mL of Pioglitazone HCl	
Pressure Drop:	67 Bar (972 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Pioglitazone HCl	7.6	1.0	0.0	1.09

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Analysis protocol for Pioglitazone HCl RS

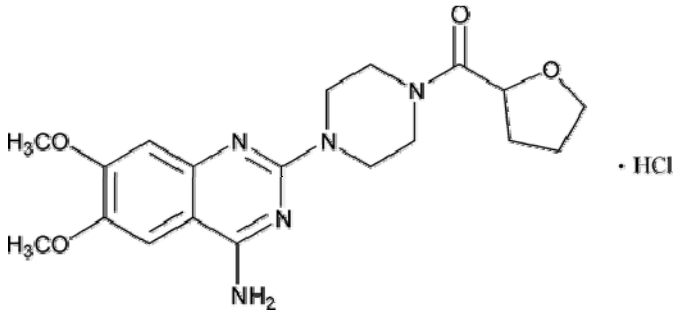
USP Method Repeatability

No	Compound	Response (Arbitrary Area Units)	Relative Standard Deviation (%)	N
1	Pioglitazone HCl	221512	0.5	6

Replicate injections of standard solution (n=6) were analyzed to determine the USP method repeatability. Sample contained 1 µg/mL of Pioglitazone HCl

Terazosin HCl

USP Method Terazosin HCl Assay



Original Manufacturer: Abbott (Patent expired 1997)

Brand Name: Hytrin, Zayasel, Blavin, Flumarc, Fosfomic, Hytracin, Hytrinex, Itrin, Urodie, Vasomet, Vicard

Manufacturers: Abbott, Apotex inc, Cadista, Ivax pharmaceuticals, Mylan, Ranbaxy, Sandoz, Teva

Terazosin is a selective alpha 1 antagonist used for treatment of symptoms of an enlarged prostate (BPH). It also acts to lower the blood pressure, and is therefore a drug of choice for men with hypertension and prostate enlargement.

It works by blocking the action of adrenaline on smooth muscle of the bladder and the blood vessel walls.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Terazosin Hydrochloride

USP34 – NF29 S1

USP Columns:

ZORBAX RX-C8 Limit of 1-[(tetrahydro-2-furanyl)carbonyl]piperazine. 4.6 mm x 25 cm, Agilent
ZORBAX RX-C8 Assay and Related Compounds 4.6 mm x 25 cm, Agilent.

Equivalent Column:

Purospher®STAR RP-8 endcapped 250x4.6 mm, 5 µm (1.51454.0001)

Recommended Solvents and Reagents:

Acetonitrile	isocratic grade for liquid chromatography LiChrosolv®	(1.14291)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	(1.15333)

Sodium citrate dihydrate	Use ACS reagent grade
Citric acid (anhydrous)	Use Anhydrous Citric Acid (USP monograph).

USP Standards

Terazosin Hydrochloride (200 mg)	USP Product Number:1643452
Terazosin Related Compound A (50 mg)	USP Product Number:1643463
Terazosin Related Compound B (50 mg)	USP Product Number: 1643474
Terazosin Related Compound C (50 mg)	USP Product Number: 1643485

USP Method Terazosin HCl Assay

pH 3.2 Citrate buffer

Dissolve 12.0 g of sodium citrate dihydrate and 28.5 g of anhydrous citric acid in 1.95 L of water. Adjust with anhydrous citric acid or sodium citrate to a pH of 3.2 ± 0.1. Dilute with water to 2.0 L, and mix.

Mobile phase

Prepare a filtered and degassed mixture of pH 3.2 Citrate buffer and acetonitrile (1685:315).
Make adjustments if necessary (*see System Suitability under Chromatography 621*).

Standard stock preparation

Dissolve an accurately weighed quantity of USP Terazosin Hydrochloride RS in Mobile phase, and dilute with Mobile phase to obtain a solution having a known concentration of about 0.5 mg per mL.

Standard preparation

Transfer 10.0 mL of Standard stock preparation to a 50-mL volumetric flask, and dilute with Mobile phase to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with Mobile phase to volume; and mix.

Assay stock preparation

Transfer about 100 mg of Terazosin Hydrochloride, accurately weighed, to a 200-mL volumetric flask; dissolve in and dilute with Mobile phase to volume; and mix.

Assay preparation

Transfer 10.0 mL of Assay stock preparation to a 50-mL volumetric flask, dilute with Mobile phase to volume, and mix. Transfer l0.0 mL of this solution to a l00-mL volumetric flask, dilute with Mobile phase to volume, and mix.

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The column temperature is maintained at about 30. The flow rate is about 1.0 mL per minute. Chromatograph the Mobile phase, and record the peak responses as directed for Procedure: ensure that there are no significant interfering peaks. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure:
the column efficiency is not less than 12,000 theoretical plates
the tailing factor is not less than 0.9 and not more than 1.3;
the relative standard deviation for replicate injections is not more than 0.9%.

Procedure

Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms for about 45 minutes, and measure the peak responses. Calculate the quantity, in mg, of $C_{19}H_{25}N_5O_4 \cdot HCl$ in the portion of Terazosin Hydrochloride taken by the formula: $10{,}000C(r_U/r_S)$

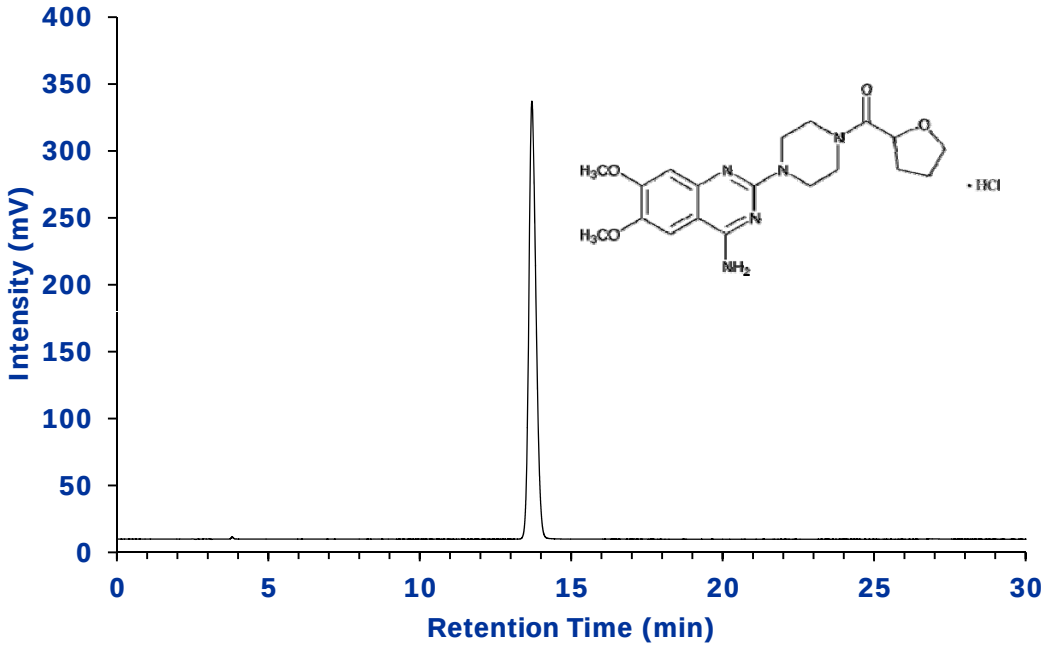
in which C is the concentration, in mg/mL, of USP Terazosin Hydrochloride RS in the Standard preparation; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

USP Method for Terazosin HCl Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-8 endcapped (5 µm) 250x4.6 mm	1.51454.0001
Injection:	20 µL	
Detection:	Shimadzu Prominence 2010, UV@220 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: Dissolve 12.0 g of sodium citrate dihydrate and 28.5 g of anhydrous citric acid in 1.95 L of water. Adjust with anhydrous citric acid or sodium citrate to a pH of 3.2 ± 0.1. Dilute with water to 2.0 L, and mix. Thereafter mix acetonitrile and buffer (315:1685)	
Temperature:	30° Celsius	
Diluent	Mobile phase	
Sample:	100 ppm of Terazosin HCl	
Pressure Drop:	146 Bar (2117 psi)	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Theoretical Plates (N)
1	Terazosin	13.7	1.2	15688

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com