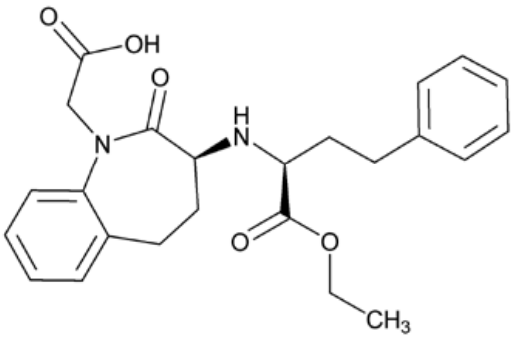
Benazepril

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

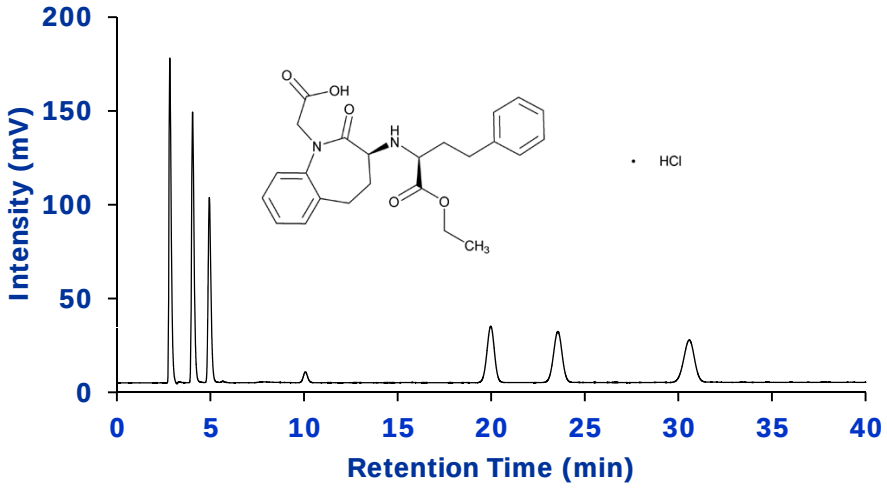
In addition to not exceeding the limits for benazepril related compounds in Table 1, not more than 0.1% of any other single impurity is found; [note—For calculating any other single unspecified impurity, C_S is the concentration of the USP Benazepril Hydrochloride RS in the Standard solution.] and not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