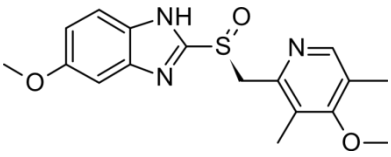
Esomeprazole (USP)



Esomeprazole is the S-enantiomer of omeprazole.

Esomeprazole is a proton pump inhibitor and reduces acid secretion through inhibition of the H+ / K+ ATPase in gastric parietal cells. By inhibiting the functioning of this transporter, the drug prevents formation of gastric acid. It is used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal reflux disease, and Zollinger-Ellison syndrome.

Common commercial brand names: Nexium, Essocam, Esomezol
Esomeprazole was developed by AstraZeneca.
Sales in 2010 were $4.9 billion globally.
Patent expired in 2014

We have followed the experimental conditions in USP37-NF32 for Esomeprazole magnesium and Esomeprazole magnesium delayed release capsules monographs.

Identification – FTIR (197K)
Identification – AAS (content of magnesium)
Assay and Related Substances – HPLC and UHPLC (both isocratic and gradient methods)
Karl Fischer – water content
Dissolution

Assay and Related Substances (RS) as well as dissolution testing have been carried out with HPLC using RP-8 and RP-18 endcapped columns with both particulate and monolithic backbones. Some of the methods are isocratic and were scaled to UHPLC settings relative to the prescribed HPLC column. Since the situation with monolithic columns is similar to that with core shell columns it is possible to make adjustments using the calculation of N and to keep this within −25% to +50%, relative to the prescribed column (see page 9-14).
We transferred the dissolution testing method for Esomeprazole magnesium delayed release capsules to a monolithic column. The new method is three times faster, having improved chromatographic resolution, lower column backpressure, and still meeting all method performance criteria.

Esomeprazole Magnesium (USP)

Definition:

Esomeprazole Magnesium contains NLT 98.0% and NMT 102.0% of $C_{34}H_{36}MgN_6O_6S_2$, calculated on the anhydrous basis.

Identification

-A. INFRARED ABSORPTION <197K> **FTIR**

-B. The sample solution, prepared and tested as directed in the test for Content of Magnesium, exhibits a significant absorption at 285.2 nm. **AAS**

Assay: **HPLC**

-Procedure:

Solution A: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

Solution B: Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.

Mobile phase: Acetonitrile and Solution A (7:13)

Standard solution: Transfer 10 mg of USP Omeprazole RS to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of Solution B, and dilute with water to volume.
[Note—This solution contains 0.05 mg/mL of omeprazole.]

Sample solution: Transfer 10 mg of Esomeprazole Magnesium to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of Solution B, and dilute with water to volume.
[Note—This solution contains 0.05 mg/mL of esomeprazole magnesium.]

Chromatographic system: (See Chromatography 621, System Suitability.)

Detector: UV 280 nm
Column: 4.0-mm × 12.5-cm or a 4.6-mm × 15-cm; 5 µm packing L7.
[Note—Alternatively, a 3.9-mm × 15-cm column that contains 4 µm packing L1 may be used.]
Flow rate: 1 mL/min
Injection size: 20 µL
We have used a Purospher® STAR RP-8 endcapped (5 µm) 150x4.6 mm (1.51453.0001) for HPLC analysis

Esomeprazole Magnesium (USP)

System suitability
Sample: Standard solution
Suitability requirements: Column efficiency: NLT 2000 theoretical plates
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of $C_{34}H_{36}MgN_6O_6S_2$ in the portion of Esomeprazole Magnesium taken:

Result = (rU/rS) × (CS/CU) × [Mr1/(2 ×Mr2)] × 100

rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of omeprazole in the Standard solution (mg/mL)
CU = concentration of Esomeprazole Magnesium in the Sample solution (mg/mL)
Mr1 = molecular weight of esomeprazole magnesium, 713.12
Mr2 = molecular weight of omeprazole, 345.42

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

Content of Magnesium AAS

Lanthanum solution: Transfer 58.7 g of lanthanum oxide into a 1000-mL volumetric flask, wet the substance with some water, and dissolve by cautious addition of 250 mL of hydrochloric acid in 20- to 30-mL portions, cooling between the additions. Add water while stirring, cool to room temperature, and dilute with water to volume.
[Note—Store the solution in a plastic bottle.]

Standard stock solution: 1000 µg/mL of magnesium in water, from a commercially prepared atomic absorption standard solution. *[Note—Store the solution in a plastic bottle.]*

Standard solution A: Transfer 10.0 mL of Standard stock solution to a 500-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and dilute with water to volume. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume.
[Note—This solution contains 2 µg/mL of magnesium.]

Standard solution B: Combine 5.0 mL of Standard solution A and 4.0 mL of Lanthanum solution, and dilute with water to 100.0 mL (0.1 µg/mL).

Esomeprazole Magnesium (USP)

Standard solution C: Combine 10.0 mL of Standard solution A and 4.0 mL of Lanthanum solution, and dilute with water to 100.0 mL (0.2 µg/mL).
Standard solution D: Combine 15.0 mL of Standard solution A and 4.0 mL of Lanthanum solution, and dilute with water to 100.0 mL (0.3 µg/mL).
Standard solution E: Combine 20.0 mL of Standard solution A and 4.0 mL of Lanthanum solution, and dilute with water to 100.0 mL (0.4 µg/mL).
Standard solution F: Combine 25.0 mL of Standard solution A and 4.0 mL of Lanthanum solution, and dilute with water to 100.0 mL (0.5 µg/mL). [Note—Concentrations of the Standard solutions and the Sample solution may be modified to fit the linear or working range of the instrument. When using instruments with a linear calibration graph, the number of Standard solutions can be reduced.]
Blank solution: Transfer 4.0 mL of Lanthanum solution to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 250 mg of Esomeprazole Magnesium to a 100-mL volumetric flask, add 20 mL of 1 N hydrochloric acid, swirl until dissolved, and dilute with water to volume. Allow to stand for 30 min. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of the solution to another 100-mL volumetric flask, add 4.0 mL of Lanthanum solution, and dilute with water to volume.

Spectrometric conditions (See Spectrophotometry and Light-Scattering <851>) AAS
Mode: Atomic absorption spectrophotometer
Flame: Air–acetylene
Analytical wavelength: 285.2 nm
Analysis
Samples: Standard solution B, Standard solution C, Standard solution D, Standard solution E, Standard solution F, Blank solution, and Sample solution . Determine the concentration, Cs, in µg/mL, of magnesium in the Sample solution using the calibration graph.

Calculate the percentage of magnesium in the portion of Esomeprazole Magnesium taken:

Result = (CS/CU) × (100/(100 F)) × 100

CS = content of magnesium in the Sample solution as calculated above (µg/mL)
CU = concentration of Esomeprazole Magnesium in the Sample solution (µg/mL)
F = content of water in Esomeprazole Magnesium, as determined in Specific Tests, Water Determin. (%)

Acceptance criteria: 3.30%–3.55%, on anhydrous basis

Esomeprazole Magnesium (USP)

IMPURITIES - Organic Impurities - Procedure 1 **HPLC**

Solution A: 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

Mobile phase: Acetonitrile and Solution A (11:29).
[Note—To improve the resolution, the composition may be changed to 1:3, if necessary.]

System suitability solution: 1 mg of USP Omeprazole RS and 1 mg of USP Omeprazole Related Compound A RS in 25 mL of Mobile phase.
[Note—Omeprazole Related Compound A is omeprazole sulfone.]

Sample solution: 4 mg of Esomeprazole Magnesium in 25 mL of Mobile phase.
[Note—Prepare this solution fresh.]

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 280 nm
Column: 4.0-mm × 12.5-cm or a 4.6-mm × 15-cm; 5 µm packing L7.
[Note—Alternatively, a 3.9-mm × 15-cm column that contains 4 µm packing L1 may be used.]
Flow rate: 0.8–1 mL/min
Injection size: 50 µL

System suitability
Sample: System suitability solution
[Note—For relative retention times, see Impurity Table below]

Name	RRT	Acceptance Criteria - NMT (%)
Omeprazole N-oxide (1)	0.45	0.1
Omeprazole sulfone (2) Omeprazole RS A	0.8	0.2
Any other individual impurities	-	0.1
Omeprazole RS	1.0	-
(1) 4-Methoxy-2-[[(RS)-(5-methoxy-1H-benzimidazol-2-yl)sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.		
(2) 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1H-benzimidazole		

Esomeprazole Magnesium (USP)

Suitability requirements
Resolution: NLT 3 between omeprazole related compound A and omeprazole
Analysis
Sample: Sample solution
Record the chromatogram for at least 4.5 times the retention time of the omeprazole peak, and measure the peak responses. Identify the impurities based on the retention times shown in Impurity Table 1.
Calculate the percentage of any individual impurity in the portion of Esomeprazole Magnesium taken:

Result = (rU/rT) × 100
rU = peak response for each impurity
rT = sum of all peak responses

Acceptance criteria: Individual impurities: See Impurity Table. Total impurities: NMT 0.5%

Procedure 2: Enantiomeric Purity
- This test could not be performed due to unavailability of a suitable chiral column"

Water Determination — Karl Fischer

Method I <921>: 6.0%–8.0%

Color of Solution
Sample solution: 20 mg/mL of Esomeprazole Magnesium in methanol, filtered
Analysis: Determine the absorbance of this solution at 440 nm, in 1-cm cells, using methanol as the blank.

Acceptance criteria: NMT 0.2

ADDITIONAL REQUIREMENTS
Packaging and Storage: Preserve in tight containers, protected from light. Store at room temperature.
USP Reference Standards 11
USP Esomeprazole Magnesium RS
USP Omeprazole RS
USP Omeprazole Related Compound A RS
Omeprazole sulfone, 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1H-benzimidazole.

Esomeprazole Magnesium (USP)

Recommended Merck Millipore products:

FTIR – Identification (197K)
Potassium bromide for IR spectroscopy Uvasol® (1.04907)
KF – Water Determination (921 –Ia)
CombiTitrant 5 one-component reagent for volumetric KF titration 1 ml = ca. 5 mg H2O apura® 1.88005
CombiSolvent methanol-free for volumetric KF titration with one component reagents apura® 1.88008

AAS - Content of Magnesium
Lanthanum(III) oxide for atomic absorption spectroscopy (1.10982)
Hydrochloric Acid (30% Ultrapur 1.01514)
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)

HPLC Assay and Related Substances (API)
Purospher® STAR RP-8 endcapped (5 µm) 150x4.6 mm (1.51453) for HPLC Assay and RS analysis
Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm (1.50629) for RS analysis
Chromolith® HighResolution RP-18 endcapped 100x4.6 mm (1.52022) for RS analysis
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur 106342
di-Sodium hydrogen phosphate dihydrate for analysis EMSURE® 106580
tri-Sodium phosphate dodecahydrate for analysis EMSURE® ACS,Reag. Ph Eur 106578
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur 100573
Acetonitrile (isocratic grade for liquid chromatography LiChrosolv® 1.14291
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)

HPLC Assay and Related Substances (Delayed Release Capsules)
Purospher® STAR RP-18 endcapped (5 µm) 150x4.6 mm (1.51455) for assay and dissolution testing
Chromolith® HighResolution RP-18 endcapped 100x4.6 mm (1.52022.0001)
Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm 1.50469.001 for RS analysis
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur 106342
di-Sodium hydrogen phosphate dihydrate for analysis EMSURE® 106580
Acetonitrile (isocratic grade for liquid chromatography LiChrosolv® 1.14291)
Acetonitrile (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur 1.00030
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)

Dissolution Testing
Hydrochloric acid (fuming 37% for analysis EMSURE® ACS,ISO,Reag. Ph Eur 100317)
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur 106342
di-Sodium hydrogen phosphate dihydrate for analysis EMSURE® 106580
Sodium hydroxide solution 50% for analysis EMSURE® 158793
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)
Millex PTFE filter

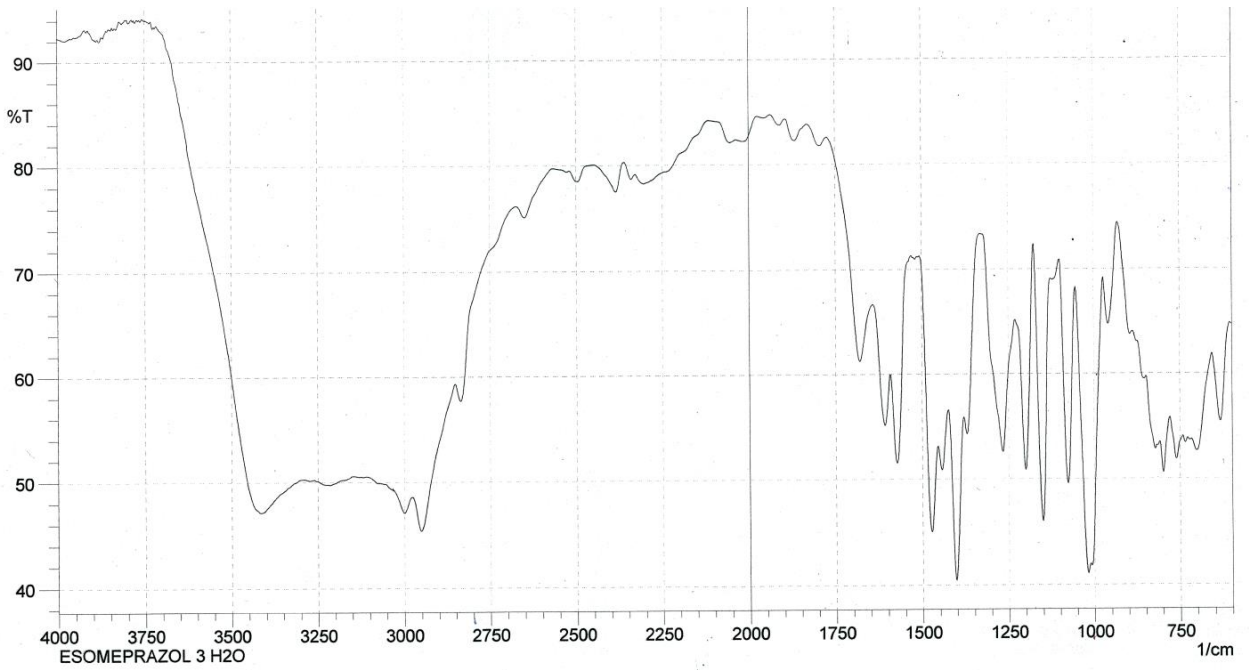
Esomeprazole Magnesium (USP)

Identification (197K)

A. INFRARED ABSORPTION <197K> FTIR

The reference 197K in a monograph signifies that the substance under examination is mixed intimately with potassium bromide.

We recommend Potassium bromide for IR spectroscopy Uvasol® (1.04907) to be used.



Esomeprazole Magnesium (USP)

B: Content of Magnesium – Atomic Absorption Spectroscopy (AAS)

Sample Solution: Transfer 250 mg of Esomeprazole Magnesium to a 100-mL volumetric flask, add 20 mL of 1 N hydrochloric acid, swirl until dissolved, and dilute with water to volume. Allow to stand for 30 min. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of the solution to another 100-mL volumetric flask, add 4.0 mL of *Lanthanum solution*, and dilute with water to volume.

Absorption at 285.2 → 0.563

Lanthanum solution: Transfer 58.7 g of lanthanum oxide into a 1000-mL volumetric flask, wet the substance with some water, and dissolve by cautious addition of 250 mL of hydrochloric acid in 20- to 30-mL portions, cooling between the additions. Add water while stirring, cool to room temperature, and dilute with water to volume. *[NOTE—Store the solution in a plastic bottle.]*

Standard stock solution: 1000 µg/mL of magnesium in water, from a commercially prepared atomic absorption standard solution. *[NOTE—Store the solution in a plastic bottle.]*

Standard solution A: Transfer 10.0 mL of Standard stock solution to a 500-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and dilute with water to volume. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. [NOTE—This solution contains 2 µg/mL of magnesium.]

We recommend Lanthanum(III) oxide for atomic absorption spectroscopy (1.10982), hydrochloric acid Ultrapur (1.01514) and Magnesium ICP standard traceable to SRM from NIST Mg(NO3)2 in HNO3 2-3% 1000 mg/l Mg Certipur® (1.70331) to be used.

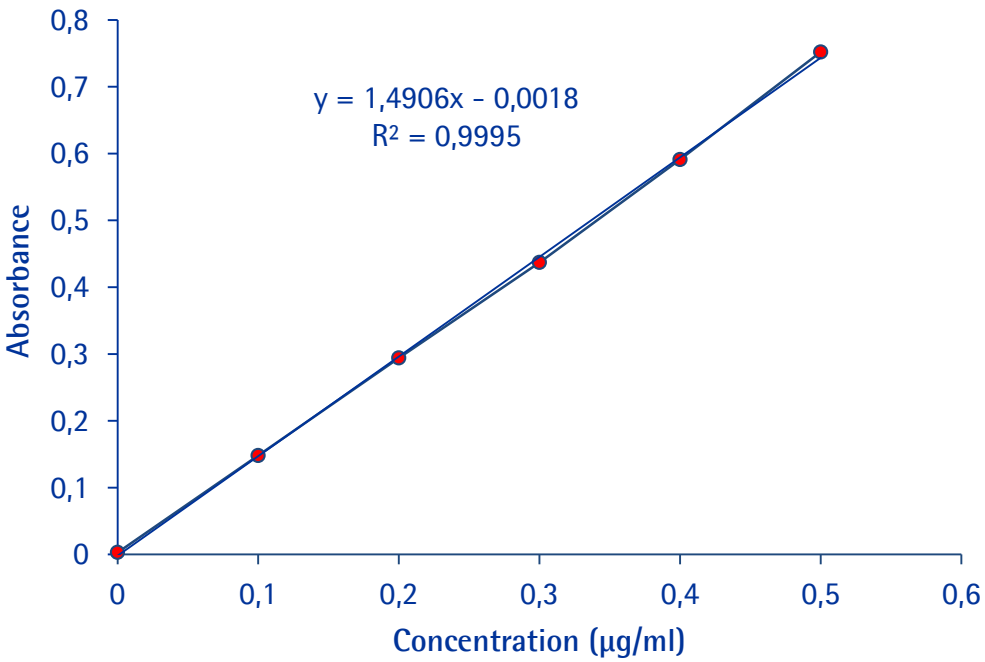
	Solution A	Lanthanum oxide solution	Dilution	Final standard concentration
Standard Solution B	5.0 ml	4.0 ml	100 ml	0.1 µg / ml
Standard Solution C	10.0 ml	4.0 ml	100 ml	0.2 µg / ml
Standard Solution D	15.0 ml	4.0 ml	100 ml	0.3 µg / ml
Standard Solution E	20.0 ml	4.0 ml	100 ml	0.4 µg / ml
Standard Solution F	25.0 ml	4.0 ml	100 ml	0.5 µg / ml

Esomeprazole Magnesium (USP)

Calibration Curve (AAS):

Concentration (µg/ml)	Absorbance
0	0.003
0.1	0.148
0.2	0.294
0.3	0.437
0.4	0.591
0.5	0.752

Absorbance for sample	0.563
Conc. calculated for sample	0.390 µg/ml



Result = (CS / CU) X (100 / (100-F)) X 100 = (0.3896/12.516) X (100/ (100 – 8.121)) X 100 = 3.39 %

The obtained value is within the acceptance criteria: 3.30%–3.55%, on anhydrous basis

Esomeprazole Magnesium (USP)

Water Determination <USP 921>

Pharmaceutical products are often characterized by complex formulations. Difficulties observed during Karl Fischer determination are often caused by the limited solubility. In some cases side reactions have to be considered. In dependence of composition and properties of the formulations, various measures are necessary for an undisturbed Karl Fischer determination.

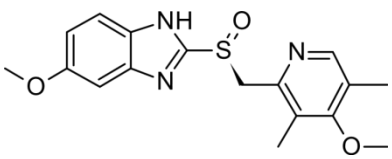
In the case of Esomeprazole the water determination can be carried out without problems according to standard methods.

In pharmaceutical guidelines (USP, Ph Eur, DAB) the Karl Fischer titration is described as common method for water determination. For some substances special procedures can be found. The determination of mass loss as method for water determination is not recommended.

Titration one component system

Titrant: apura - CombiTitrant 5 (1.88005)
One component reagent for volumetric Karl Fischer titration, 1 mL = approx. 5 mg water
Solvent: apura – CombiSolvent - methanol-free solvent for volumetric Karl Fischer titration with one component reagents; 50 ml (1.88008)

Titration parameters
Stirring time: 90 s
Default titration settings, e.g.:
I(pol) = 20 - 50 µA, U(EP) = 100 - 250 mV
Stop criterion: drift < 20 µL/min
Sample size: 0.2 g (we used Esomeprazole Magnesium RS)



Result:
Measured water content in Esomeprazole: 7,63% **(USP - requirement: 6-8%)**

Procedure
The titration medium is first placed into the titration cell and titrated dry by means of the titrant. Then the sample is added from a weighing boat (exact sample weight determination by weighing of weighing boat before and after addition) and the titration is started. For complete dissolution of the sample a stirring time of 90 seconds is recommended.

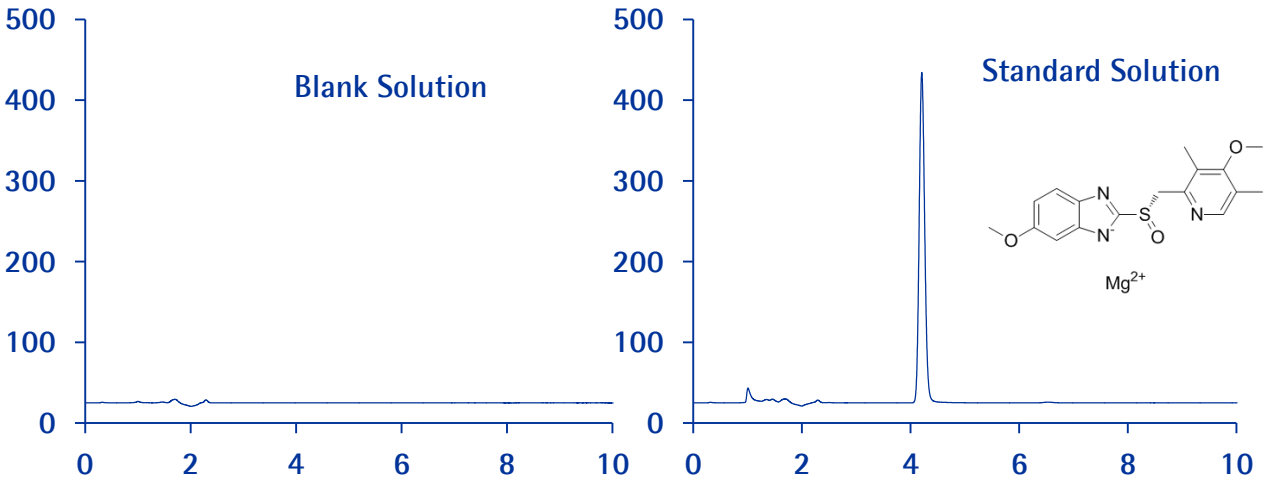
Product	P/N
CombiTitrant 5 one-component reagent for volumetric KF titration 1 ml = ca. 5 mg H2O apura®	1.88005
CombiSolvent methanol-free solvent for volumetric KF titration with one component reagents apura®	1.88008

Esomeprazole Magnesium (USP) – Assay

Purospher STAR® RP-8 endcapped HPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (5 µm) 150x4.6 mm	1.51453.0001
Injection:	20 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, and dilute with water to 1000 mL. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6. Mix acetonitrile and Solution A (7:13 v/v)	
Temperature:	25°C	
Diluent:	Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.	
Standard Solution:	Transfer 10 mg of USP Omeprazole to a 200-mL volumetric flask, and dissolve in about 10 mL methanol. Add 10 mL of Solution B, and dilute with water to final volume.	
Sample Solution:	Transfer 10 mg of Esomeprazole Magnesium to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of Solution B, and dilute with water to final volume.	
Pressure Drop:	101 Bar (1464 psi)	



System Suitability requirement: Column efficiency: NLT 2000 theoretical plates

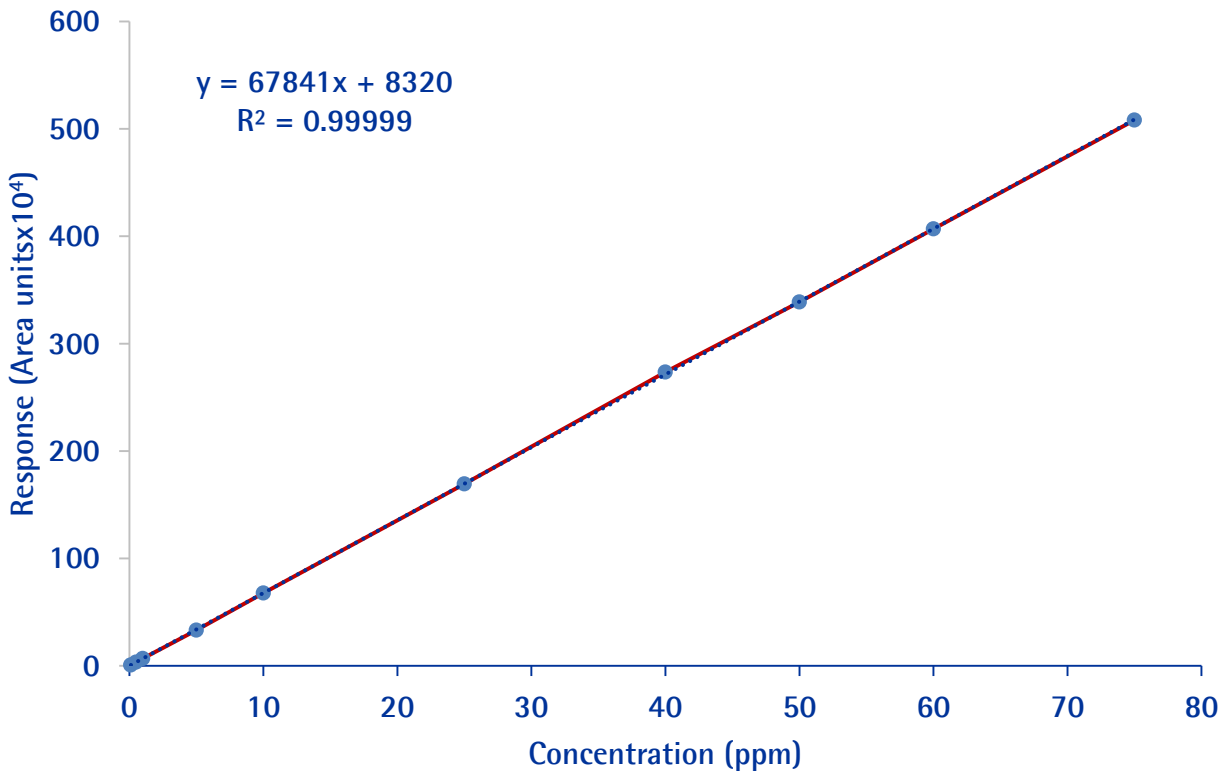
Chromatographic Data:

Compound	Retention Time (min)	Plates	Tailing Factor
Impurity A			
Omeprazole	4.2	8269	1.1

Esomeprazole Magnesium (USP)

Linearity:

Concentration (ppm)	Area Units
0.1	7273
0.5	33723
1	68763
5	331460
10	677630
25	1694075
40	2734520
50	3388150
60	4068780
75	5082225
STEYX	8320
Slope	67841
LOD	0.4
LOQ	1.2

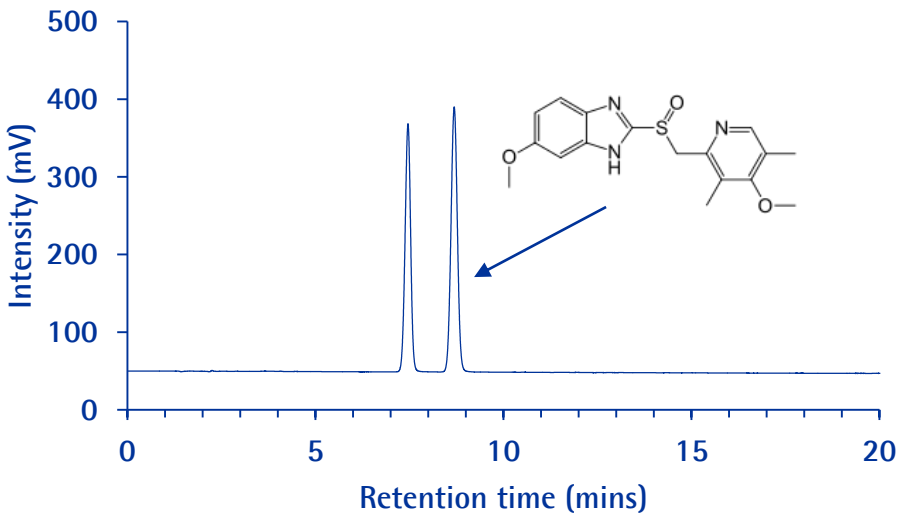


Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped HPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (5 µm) 150x4.6 mm	1.51453.0001
Injection:	50 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Solution A:	Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1000mL water. If necessary, adjust with phosphoric acid to a pH of 7.6.	
Mobile Phase :	Acetonitrile:Solution A 11:29 (v/v)	
Temperature:	Ambient	
Diluent:	Mobile phase	
SST solution:	Dissolve 1.0mg of Omeprazole standard & related compound A in 25 mL of diluent.	
Sample solution:	Dissolve 4.0mg of sample in 25 mL of diluent.	
Pressure Drop:	87 Bar (1261 psi)	



Suitability requirements
Resolution: NLT 3 between omeprazole related compound A and omeprazole
Relative retention time (RRT): 0.8 for and 1.0 for omeprazole related compound A and omeprazole, respectively

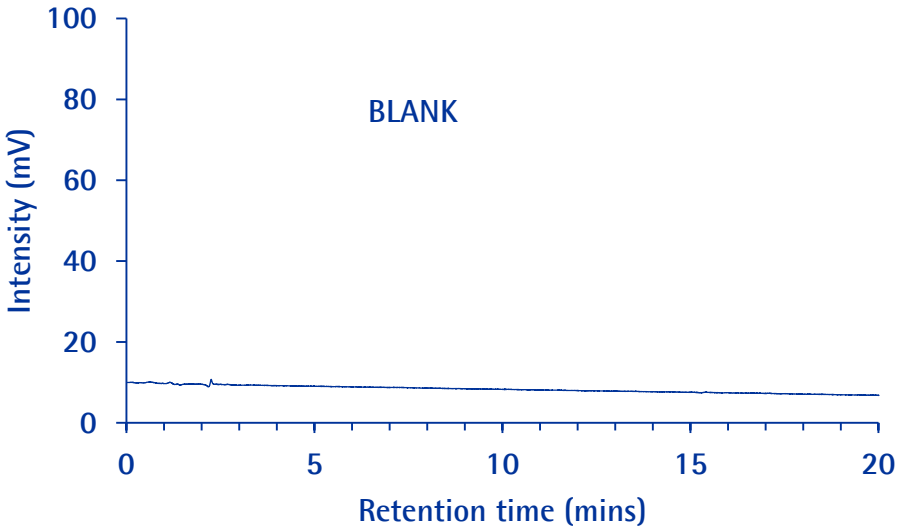
Chromatographic Data : SST solution

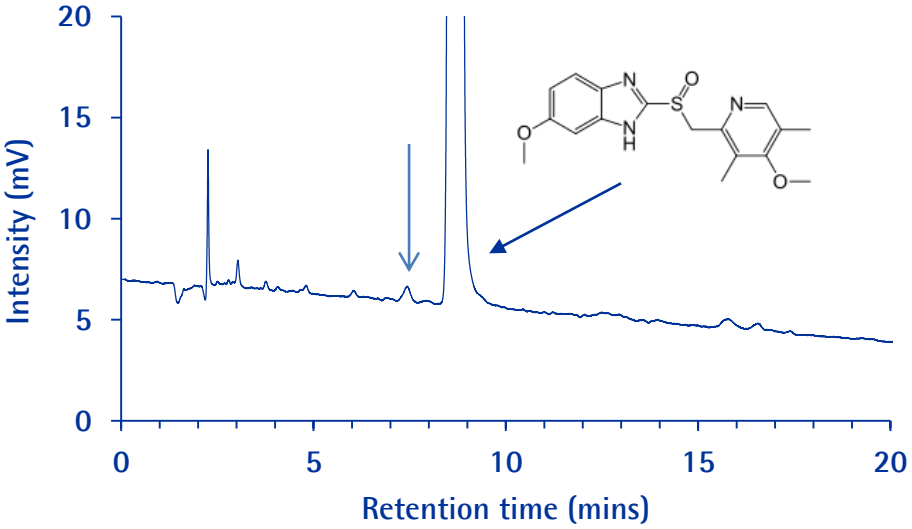
No.	Compound	Retention Time (min)	Resolution	RRT
1	Omeprazole Related compound A	7.46	-	0.85
2	Omeprazole	8.69	4.2	1.00

Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped

HPLC





Chromatographic Data : SST solution

No.	Compound	Retention Time (min)	Resolution	RRT
1	Related compound A	7.46	-	0.85
2	Esomeprazole	8.69	4.2	1.00

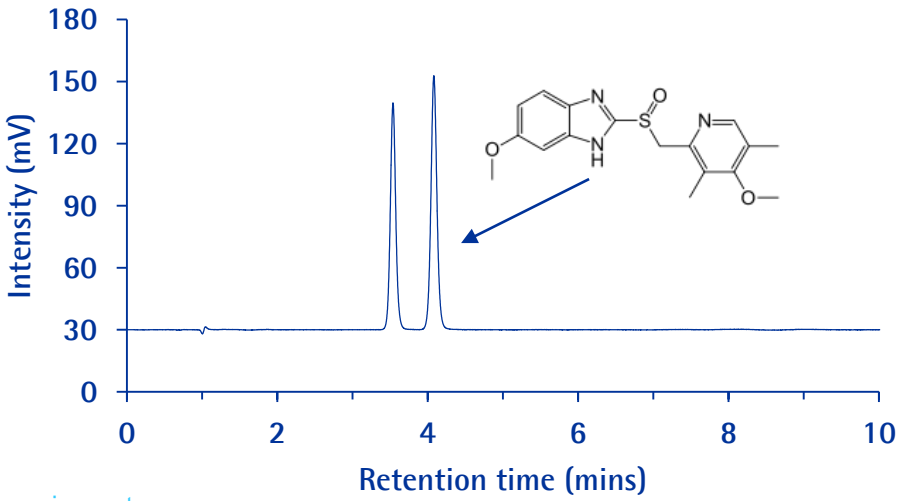
Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped

UHPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm	1.50629.0001
Injection:	5 µL	
Detection:	UV 280 nm	
Cell:	2.5 µL	
Flow Rate:	0.3 mL/min	
Solution A:	Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1000mL water. If necessary, adjust with phosphoric acid to a pH of 7.6.	
Mobile Phase :	Acetonitrile:Solution A (27:73) (v/v)	
Temperature:	Ambient	
Diluent:	Mobile phase	
SST solution:	Dissolve 1.0mg of Omeprazole standard & related compound A in 25 mL of diluent.	
Sample solution:	Dissolve 4.0mg of sample in 25 mL of diluent.	
Pressure Drop:	300 Bar (4350 psi)	



Suitability requirements
Resolution: NLT 3 between omeprazole related compound A and omeprazole
Relative retention time (RRT): 0.8 for and 1.0 for omeprazole related compound A and omeprazole, respectively

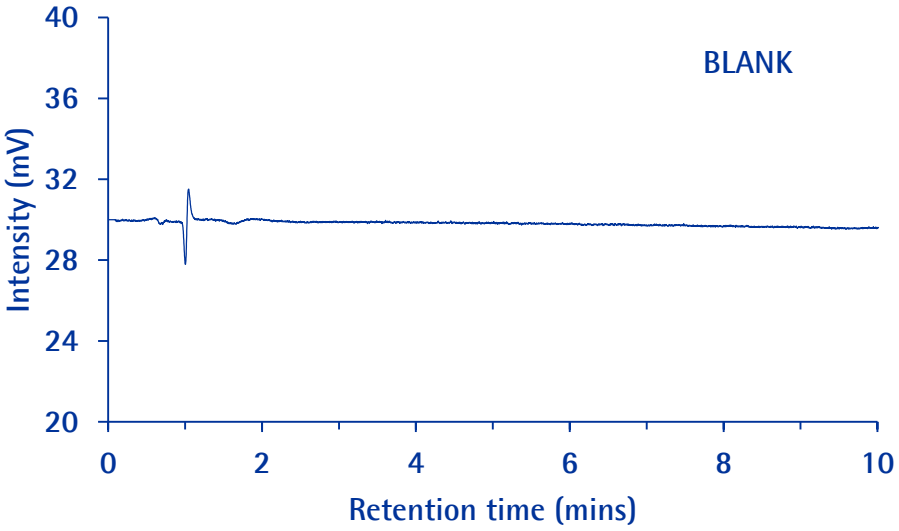
Chromatographic Data : SST solution

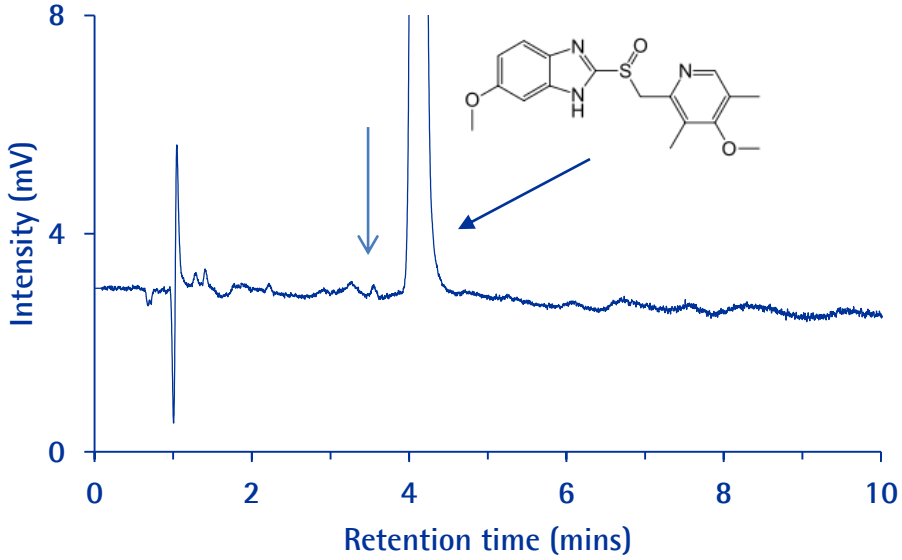
No.	Compound	Retention Time (min)	Resolution	RRT
1	Related compound A	3.5	-	0.85
2	Omeprazole	4.1	4.2	1.00

Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped

UHPLC





Chromatographic Data : SST solution

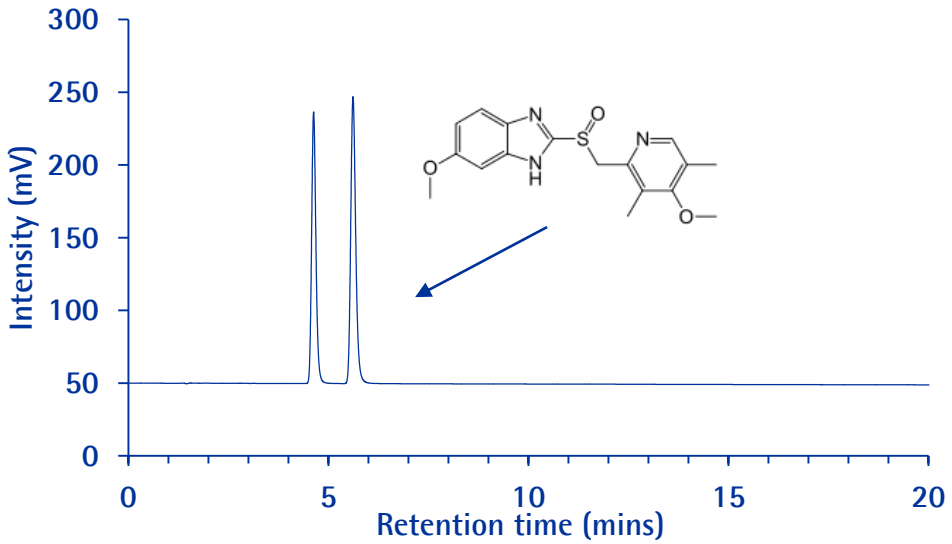
No.	Compound	Retention Time (min)	Resolution	RRT
1	Related compound A	3.5	-	0.85
2	Omeprazole	4.1	4.2	1.00

Esomeprazole Related Substances (USP)

Chromolith HighResolution® RP-18 endcapped HPLC

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Solution A:	Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1000mL water. If necessary, adjust with phosphoric acid to a pH of 7.6.	
Mobile Phase :	Acetonitrile:Solution A 25:75 (v/v)	
Temperature:	Ambient	
Diluent:	Mobile phase	
SST solution:	Dissolve 1.0mg of Omeprazole standard and related compound A in 25 mL of diluent.	
Sample solution:	Dissolve 4.0mg of sample in 25 mL of diluent.	
Pressure Drop:	50 Bar (725 psi)	



Suitability requirements
Resolution: NLT 3 between omeprazole related compound A and omeprazole
Relative retention time (RRT): 0.8 for and 1.0 for omeprazole related compound A and omeprazole, respectively

Chromatographic Data : SST solution

No.	Compound	Retention Time (min)	Resolution	RRT
1	Related compound A	4.6	-	0.82
2	Omeprazole	5.6	4.7	1.00

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

Definition:
Esomeprazole Magnesium Delayed-Release Capsules contain an amount of Esomeprazole Magnesium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of esomeprazole ($C_{34}H_{36}MgN_6O_6S_2$,).

Identification
A. Enantiomeric purity – not performed as it requires a chiral column (4.0×10 mm; 5 µm packing L41)

Assay: HPLC
-Procedure:
Buffer: Prepare a pH 7.3 phosphate buffer by mixing 10.5 mL of 1.0 M monobasic sodium phosphate buffer and 60 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL.

Diluent: Prepare as directed in Identification test A.

Mobile phase: Mix 350 mL of acetonitrile and 500 mL of the Buffer. Dilute with water to 1000 mL.

Standard solution: Transfer 10 mg of USP Omeprazole RS to a 250-mL volumetric flask, and dissolve in about 10 mL of alcohol. Add 40 mL of Diluent, and dilute with water to volume. This solution contains 0.04 mg/mL of USP Omeprazole RS.

Sample stock solution: Mix the contents of NLT 20 Capsules. Transfer a portion of the Capsule content, equivalent to 20 mg of esomeprazole, to a 100-mL volumetric flask, add 60 mL of Diluent, and shake for 20 min to dissolve the pellets. Sonicate for a few minutes, if needed, to completely dissolve. Add 20 mL of alcohol, and sonicate for a few minutes. Cool, and dilute with Diluent to volume.
Pass a portion of the solution through a filter of 1 µm pore size.

Sample solution: 0.04 mg/mL of esomeprazole from the Sample stock solution in water.
Store this solution protected from light.

Chromatographic system: (See Chromatography 621, System Suitability.)
Detector: UV 302 nm
Column: 4.6-mm × 15-cm; 5 µm packing L1.
Flow rate: 1 mL/min
Injection size: 20 µL

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

System suitability
Sample: Standard solution

Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) in the portion of the Capsules taken:

Result = (rU/rS) × (CS/CU) × 100

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

Acceptance criteria: 90.0%–110.0%

Dissolution <711> HPLC

Medium: 0.1 N hydrochloric acid; 300 mL. After 2 h, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 mL of 0.086 M dibasic sodium phosphate, and adjust with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05.

Apparatus 2: 100 rpm
Time: 30 min in a pH 6.8 phosphate buffer

Standard solution: Prepare a solution containing 2 mg/mL of USP Omeprazole RS in alcohol. Dilute this solution with pH 6.8 phosphate buffer to obtain a solution containing (L/1000) mg/mL, where L is the label claim, in mg/Capsule. Immediately add 2.0 mL of 0.25 M sodium hydroxide to 10.0 mL of this solution, and mix.
[Note—Do not allow the solution to stand before adding the sodium hydroxide solution.]

Sample solution: After 30 min in pH 6.8 phosphate buffer, pass a portion of the solution under test through a suitable filter. Transfer 5.0 mL of the filtrate to a suitable glassware containing 1.0 mL of 0.25 M sodium hydroxide. Mix well. Protect from light.

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

Buffer, Mobile phase, System suitability, and Chromatographic system: Proceed as directed in the Assay.

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of esomeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

Result = (rU/rS) × (CS/L) ×V× 100

rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of the Standard solution (mg/mL)
L = label claim (mg/Capsule)
V = volume of Medium, 1000 mL
Tolerances: NLT 75% (Q) of the labeled amount of esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

IMPURITIES - Organic Impurities **HPLC**

Buffer: Prepare a pH 7.6 phosphate buffer by mixing 5.2 mL of 1.0 M monobasic sodium phosphate buffer and 63 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL.
Solution A: Mix 100 mL of acetonitrile and 100 mL of the Buffer. Dilute with water to 1000 mL.
Solution B: Mix 800 mL of acetonitrile and 10 mL of the Buffer. Dilute with water to 1000 mL.

Mobile phase: See Table.

Time (min)	Solution A (%)	Solution A (%)
0	100	0
10	80	20
30	0	100
31	100	0
45	100	0

Diluent: Prepare as directed in Identification test A.
System suitability stock solution: 1 mg/mL each of USP Omeprazole RS and USP Omeprazole Related Compound A RS in methanol
System suitability solution: 1 µg/mL each of USP Omeprazole RS and USP Omeprazole Related Compound A RS from System suitability stock solution, in a mixture of Diluent and water (1:4)
Sample solution: Transfer a portion of the powdered pellets (about 80–90 mg), from the Capsule content, to a 200-mL volumetric flask, add 20 mL of methanol, and shake for 30 s. Add 40 mL of Diluent, shake for 30 s by hand, and sonicate for a few minutes. Cool, and dilute with water to volume.
Pass a portion of the solution through a filter of 0.45 µm pore size.
[Note—The solution is stable for 3 h if stored protected from light.]

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 302 nm
Column: 4.6-mm × 10-cm; 3 µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Sample: System suitability solution
[Note—See Table 2 for the relative retention times.]

Suitability requirements
Resolution: NLT 2.5 between omeprazole related compound A and omeprazole

Analysis
Sample: Sample solution
Calculate the percentage of any individual impurity in the portion of the Capsules taken:

Result = (rU/rT) × 100

rU = peak response for each impurity
rT = sum of all peak responses

Acceptance criteria: See Table.

Name	RRT	Acceptance criteria, NMT (%)
Omeprazole sulfone[a]	0.93	0.5
Omeprazole	1.0	-
Any other individual impurity	-	0.2
Total impurities	-	2

ADDITIONAL REQUIREMENTS
Packaging and Storage: Preserve in tight containers. Store at room temperature.

USP Reference Standards
USP Omeprazole RS
USP Omeprazole Related Compound A RS = Omeprazole sulfone =
= 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfonyl]-1H-benzimidazole. ($C_{17}H_{19}N_3O_4S$)

Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm 1.50469.001

Injection: 20 µL

Detection: UV 302 nm

Cell: 10 µL

Flow Rate: 1.0 mL/min

Mobile Phase: Buffer: Prepare a pH 7.6 phosphate buffer by mixing 5.2 mL of 1.0 M monobasic sodium phosphate buffer and 63 mL of 0.5 M dibasic sodium phosphate buffer diluting with water to 1000 mL.

Solution A: Mix 100 mL of acetonitrile and 100 mL of the Buffer. Dilute with water to 1000 mL.
Solution B: Mix 800 mL of acetonitrile and 10 mL of the Buffer. Dilute with water to 1000 mL.

Gradient: See table

Temperature: 25°C

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
10	80	20
30	0	100
31	100	0
45	100	0

Diluent: Dissolve 5.24 g of tribasic sodium phosphate dodecahydrate in water.
Add 110 mL of 0.5 M dibasic sodium phosphate solution, and dilute with

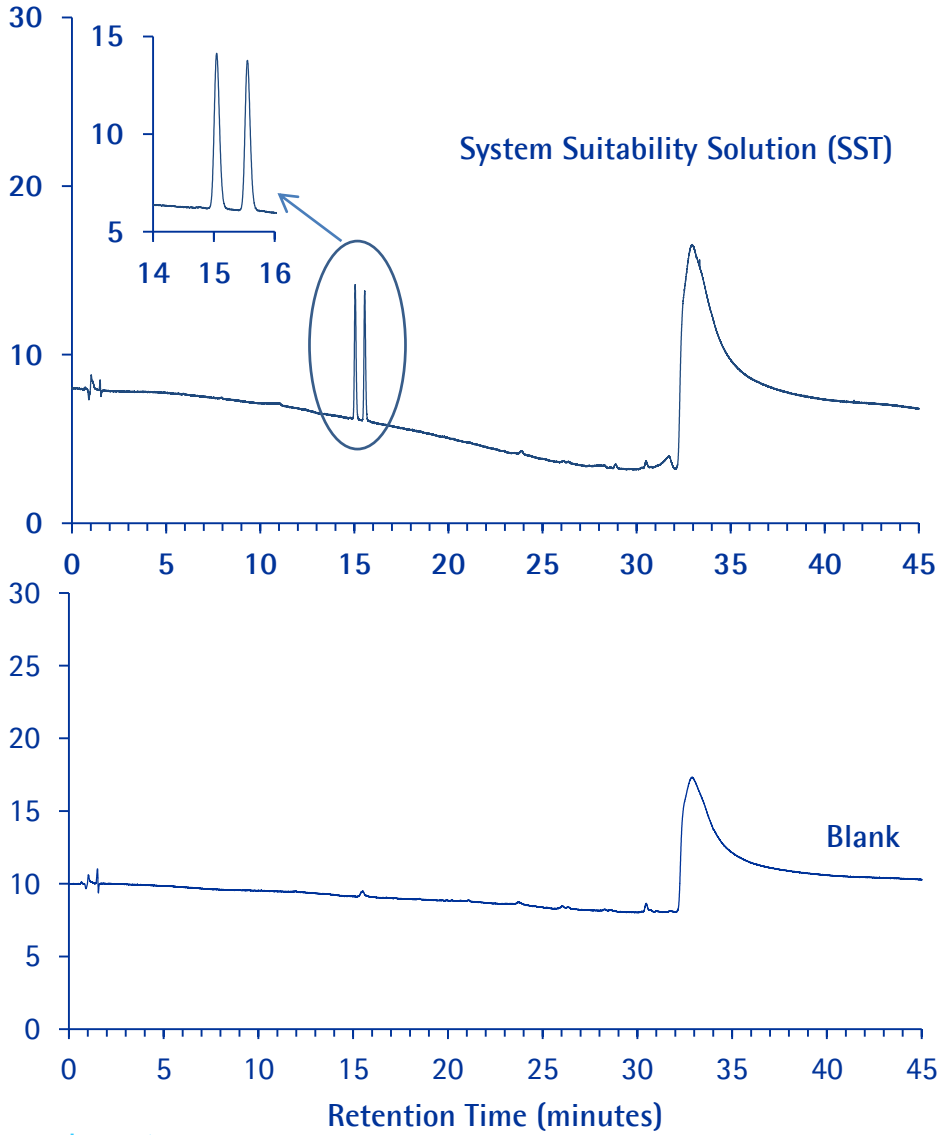
Standard Solution: 1 µg/mL each of USP Omeprazole and USP Omeprazole Related Compound A in methanol

Sample Solution: Transfer a portion of the powdered pellets (about 80–90 mg), from the capsule content, to a 200-mL volumetric flask, add 20 mL of methanol shake for 30 s. Add 40 mL of Diluent, shake for 30 s by hand, and sonicate for a few minutes. Cool, and dilute with water to volume. Pass a portion of the solution through a filter of 0.45-µm pore size.

Pressure Drop: 149 Bar to 95 Bar (2160 - 1378 psi)

Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities



Suitability requirements
Resolution: NLT 2.5 between omeprazole related compound A and omeprazole
Relative retention time (RRT): 0.8 for and 1.0 for omeprazole related compound A and omeprazole, respectively

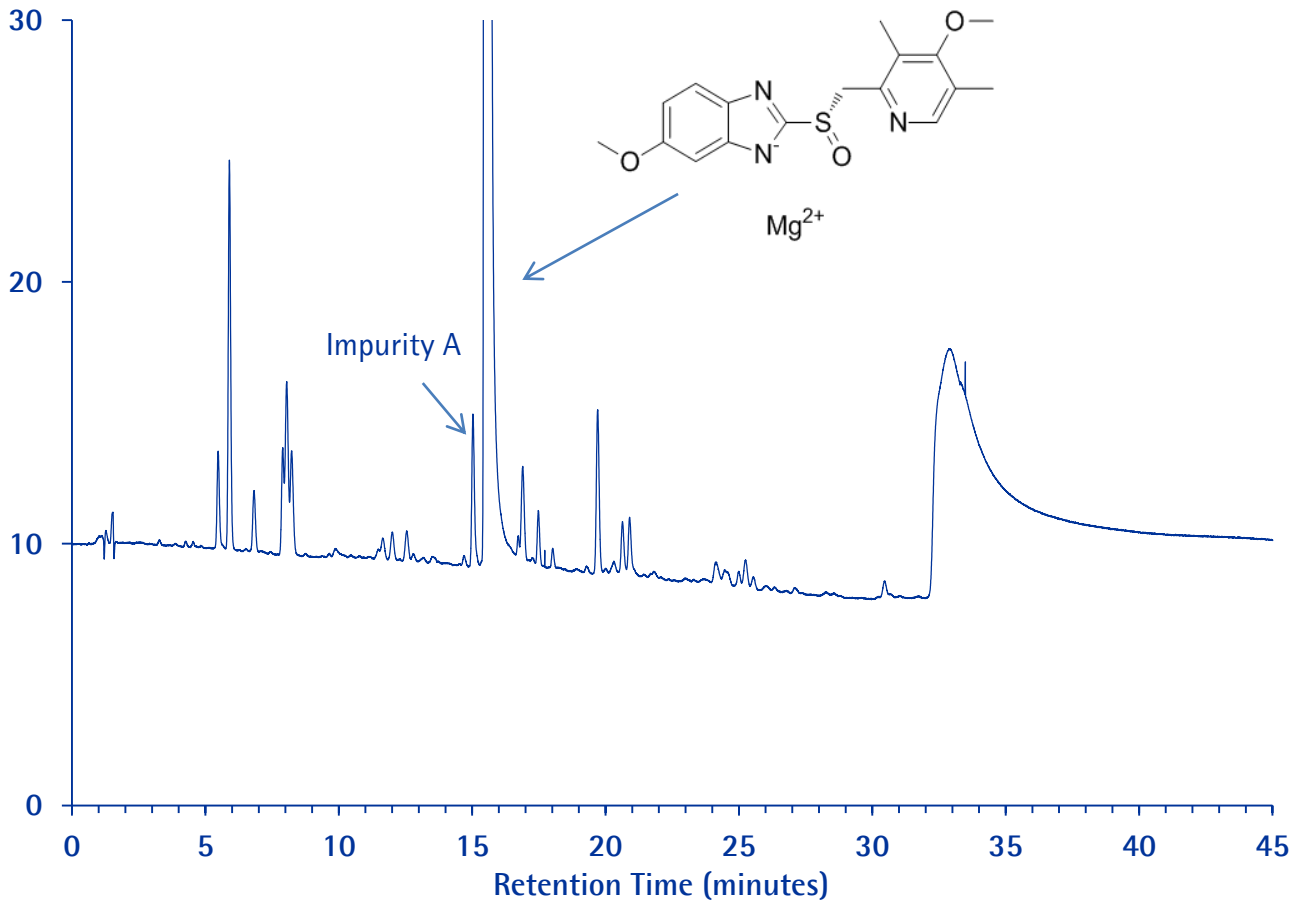
Chromatographic Data:

Compound	Retention Time (min)	RRT	Resolution	Tailing Factor
Impurity A	15.0	0.96	-	1.1
Omeprazole	15.6	1.00	3.2	1.2

Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities

Sample Analysis (delayed release capsules)



Suitability requirements
Resolution: NLT 2.5 between omeprazole related compound A and omeprazole
Relative retention time (RRT): 0.8 for and 1.0 for omeprazole related compound A and omeprazole, respectively

Chromatographic Data:

Compound	Retention Time (min)	RRT	Resolution	Tailing Factor
Impurity A	15.0	0.96	-	1.1
Omeprazole	15.6	1.0	3.2	1.2

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merckmillipore.com/perfect-solution

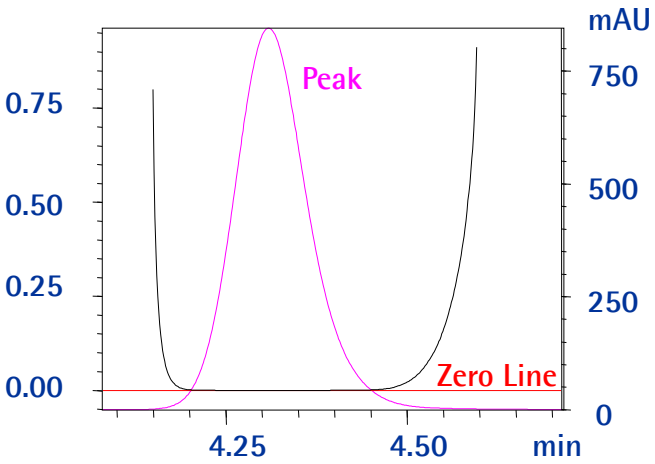
Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Dissolution

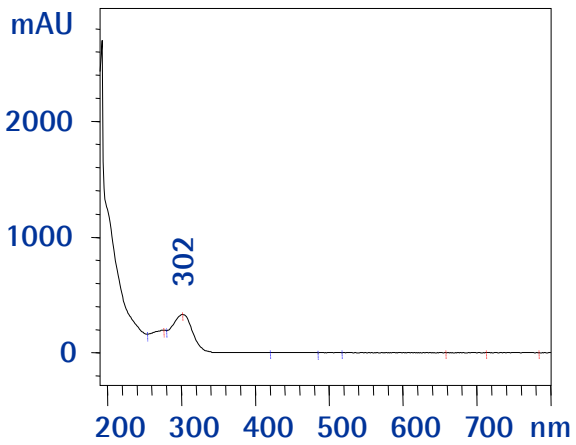
Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5 µm) 150x4.6 mm	1.51455.0008
Injection:	20 µL	
Detection:	UV 302 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Medium:	0.1 N hydrochloric acid; 300 mL. After 2 h, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 mL of 0.086 M dibasic sodium phosphate, and adjust with 2 N hydrochloric acid or 2 N sodium hydroxide if necessary, to a pH of 6.8 ± 0.05.	
Apparatus 2:	100 rpm **(Time: 30 min in a pH 6.8 phosphate buffer)**	
Mobile phase:	Buffer: Prepare a pH 7.3 phosphate buffer by mixing 10.5 mL of 1.0 M monobasic sodium phosphate buffer and 60 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL. Mix 350 mL of acetonitrile and 500 mL of the Buffer. Dilute with water to 1000 mL.	
Temperature:	25°C	
Diluent:	Dissolve 5.24 g of tribasic sodium phosphate dodecahydrate in water. Add 110 mL of 0.5 M dibasic sodium phosphate solution, and dilute with water to 1000 mL.	
Standard Solution:	Transfer 10 mg of USP Omeprazole RS to a 250-mL volumetric flask, and dissolve in about 10 mL of alcohol. Add 40 mL of Diluent, and dilute with water to volume.	
Sample Solution:	After 30 min in pH 6.8 phosphate buffer, pass a portion of the solution under test through a suitable filter. Transfer 5.0 mL of the filtrate to a suitable glassware containing 1.0 mL of 0.25 M sodium hydroxide. Mix well. Protect from light.	
Pressure Drop:	149 Bar (2160 psi)	

Peak Purity

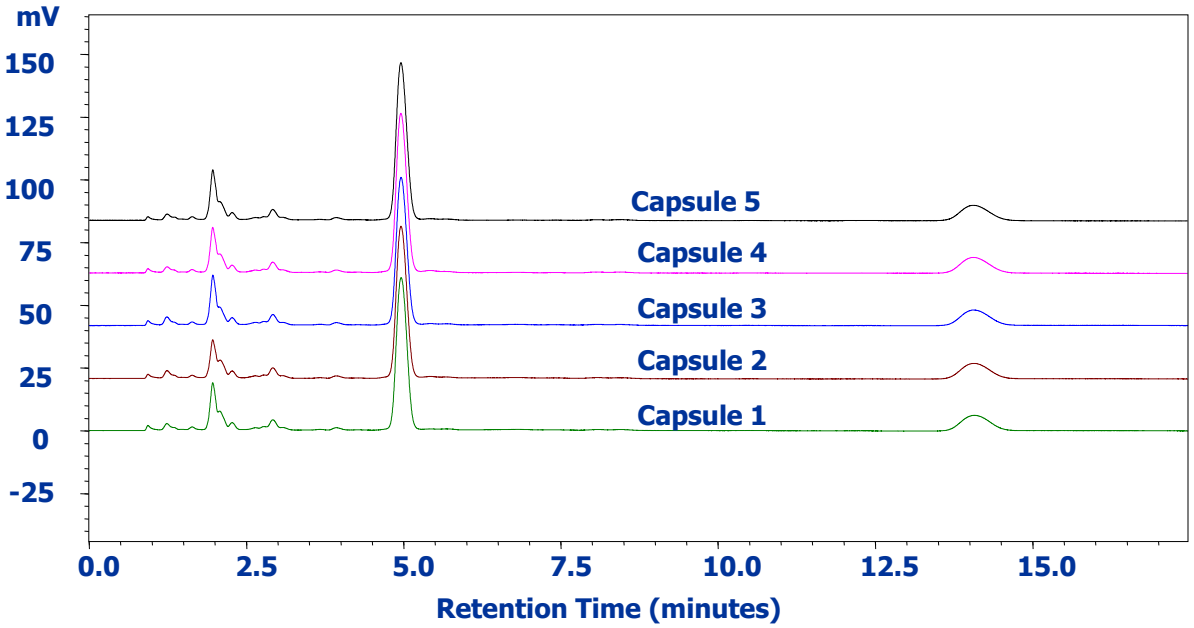


UV Analysis



Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities



Sample (area units)	Standard (area units)	[Standard solution] (mg/ml)	Label claim (mg/capsule)	Media volume (ml)	Dissolution (%)
318234	357635	0.041	40	1000	91.2
312926					89.7
316158					90.6
313776					89.9
311351					89.2
Average					90.1

Calculate the percentage of esomeprazole dissolved: Result = (rU/rS) × (CS/L) ×V× 100 = 90.1%

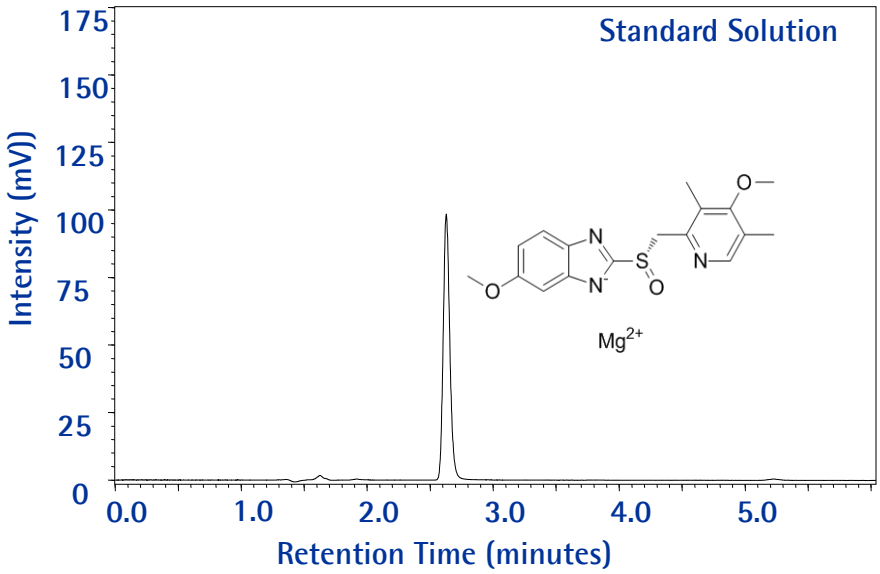
rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of the Standard solution (mg/mL)
L = label claim (mg/Capsule)
V = volume of Medium, 1000 mL
Acceptance criteria: NLT 75% of the claimed esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

Esomeprazole Magnesium Capsules (USP)

Chromolith® RP-18 endcapped – Related Impurities

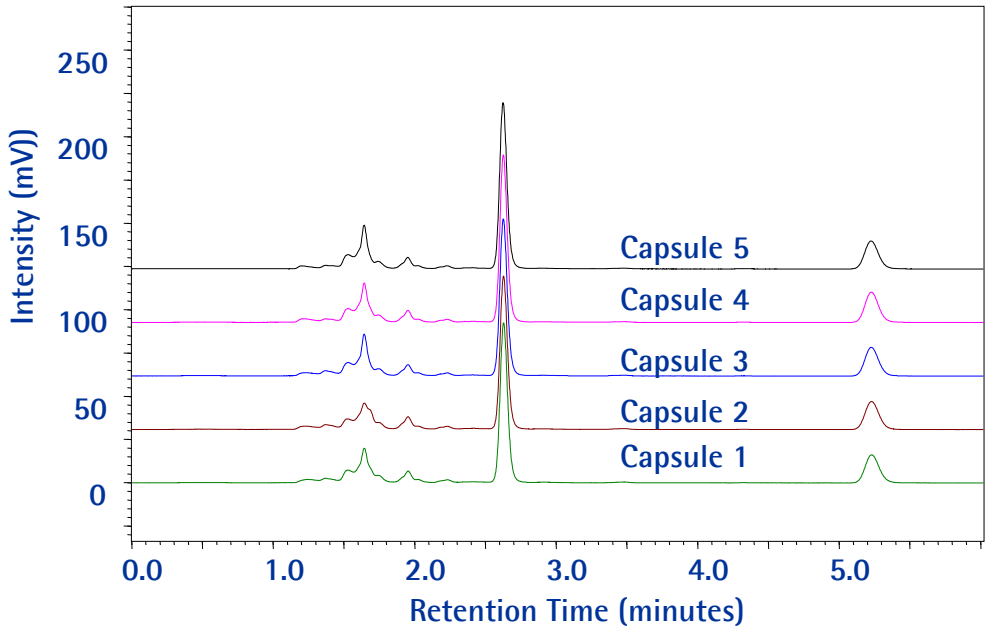
Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	5 µL (*linear scaling=13 µL but the efficiency is higher than with particle packed column so we reduced it further*)	
Detection:	UV 302 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Medium:	0.1 N hydrochloric acid; 300 mL. After 2 h, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 mL of 0.086 M dibasic sodium phosphate, and adjust with 2 N hydrochloric acid or 2 N sodium hydroxide if necessary, to a pH of 6.8 ± 0.05.	
Apparatus 2:	100 rpm (Time: 30 min in a pH 6.8 phosphate buffer)	
Mobile phase:	Buffer: Prepare a pH 7.3 phosphate buffer by mixing 10.5 mL of 1.0 M monobasic sodium phosphate buffer and 60 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 mL. Mix 350 mL of acetonitrile and 500 mL of the Buffer. Dilute with water to 1000 mL.	
Temperature:	25°C	
Diluent:	Dissolve 5.24 g of tribasic sodium phosphate dodecahydrate in water. Add 110 mL of 0.5 M dibasic sodium phosphate solution, and dilute with water to 1000 mL.	
Standard Solution:	Transfer 10 mg of USP Omeprazole RS to a 250-mL volumetric flask, and dissolve in about 10 mL of alcohol. Add 40 mL of Diluent, and dilute with water to volume.	
Sample Solution:	After 30 min in pH 6.8 phosphate buffer, pass a portion of the solution under test through a suitable filter. Transfer 5.0 mL of the filtrate to a suitable glassware containing 1.0 mL of 0.25 M sodium hydroxide. Mix well. Protect from light.	
Pressure Drop:	75 Bar (1080 psi)	



Esomeprazole Magnesium Capsules (USP)

Chromolith® RP-18 endcapped – Related Impurities



Sample (area units)	Standard (area units)	[Standard solution] (mg/ml)	Label claim (mg/capsule)	Media volume (ml)	Dissolution (%)
671494	751234	0.041	40	1000	91.6
656845					89.6
665258					90.8
658643					89.9
655000					89.3
Average					90.2

Calculate the percentage of esomeprazole dissolved: Result = (rU/rS) × (CS/L) ×V× 100 = 90.2%

rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of the Standard solution (mg/mL)
L = label claim (mg/Capsule)
V = volume of Medium, 1000 mL

Acceptance criteria: NLT 75% of the claimed esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

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