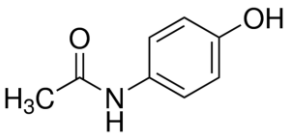
Complete solutions for Dissolution Testing

Monograph methods for Tablets and Capsules
2015-2

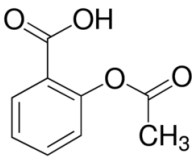
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Content

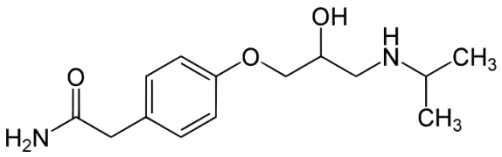
Molecular Structures



Acetaminophen



Aspirin
(acetylsalicylic acid)



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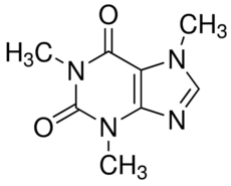

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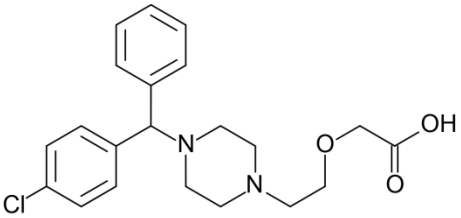

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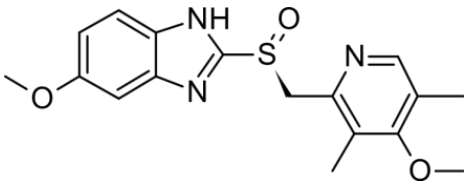
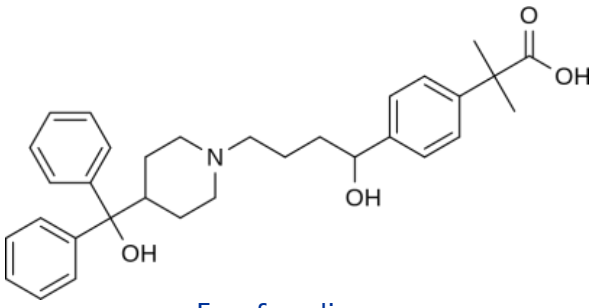


Esomeprazole



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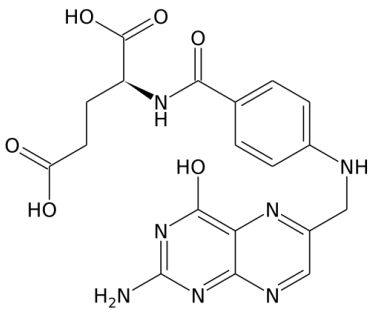
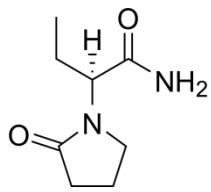
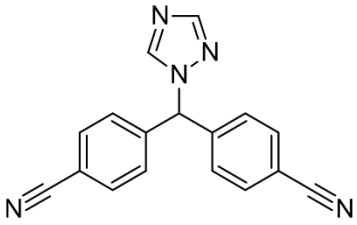


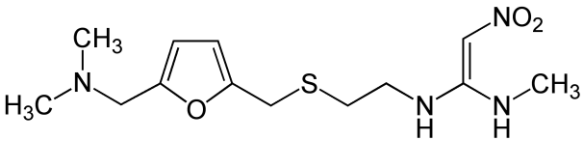
Folic acid



Levetiracetam



Letrozole



Ranitidine

Application Index

Introduction

Throughout the drug development cycle dissolution test is carried out and goals of dissolution changes as the drug moves through different stages of the development (see flow chart, page 6.) The effectiveness of different drug dosage forms relies on the drug dissolving in the body fluids prior to absorption into the systemic circulation. It is crucial to optimize the rate of dissolution; i.e. the amount of drug available to the body over a period of time (bioavailability). Deviations can mean that the treatment is ineffective and at worst potentially dangerous (toxic overdose) for the patient.

During early drug discovery stage, when new chemical entities are being discovered against new targets, no dissolution test is carried out. As the drug moves into development, formulation development begins and goal of dissolution test at this stage is to help formulation scientists to understand how formulation affects the dissolution profile. This is even more critical for extended, modified and delayed release formulations, as they need to release drug in a specific fashion. Once the drug development moves on to approval stage, dissolution test data becomes an integral part of NDA documentation and FDA requirements. As the drug moves into production, dissolution test becomes a QC test and helps give a clear understanding how changes in processing and manufacturing can affect the dissolution profile. For instance, what happens when a new lot of excipient is used in the formulation or what happens to the dissolution profile when a new supplier for a certain excipient needs to be selected. The dissolution test is now a key part of the QC process determining whether a lot passes or fails.

Finally as the drug moves into the generic arena, the dissolution test is one test which is essential to prove that the generic drug is bio-equivalent to the original patent protected drug. During all these stages, scientists are always looking for ways to find a correlation between the in-vitro dissolution test and in-vivo drug concentration profile (IVIVC) to establish the dissolution test as a surrogate for human studies, as stated by the Food and Drug Administration (FDA). Analytical data from drug dissolution testing are sufficient in many cases to establish safety and efficacy of a drug product without in vivo tests, following minor formulation and manufacturing changes. Thus, the dissolution testing which is conducted in dissolution apparatus must be able to provide accurate and reproducible results. Drug dissolution testing methods can, for these reasons, be found in pharmacopeial monograph methods for solid dosage forms, transdermal patches, suspensions, and suppositories.

In this compilation you will find complete solutions for dissolution testing of a few selected formulated small molecule drugs. The highlighted methods follow the current USP37-NF32 monographs, and are all compliant with USP38-NF33 and the given system suitability requirements. In all examples, we have used different high quality products from EMD Millipore; HPLC columns, solvents (including water), high purity reagents, as well as the most appropriate Millex filters.

Drug Development Cycle

• No dissolution testing	• Formulation optimization • Formulation changes	• NDA submission • FDA requirement	• Manufacturing changes • Process modifications • QC release test	• Bio-equivalence • Manufacturing changes • QC release test • USP
	• IVIVC	• IVIVC	• IVIVC	• IVIVC

IVIVC: in-vitro dissolution test and in-vivo drug concentration profile

Goals of Dissolution Testing During Drug Development Process

To generate useful information at each stage, dissolution must be:

- Robust
- Easy to optimize and scale up
- Produce consistent, accurate, reproducible data

Pharmacopeial Requirements

A monograph represents published standard methods by which the use of one or more substances is authorized. By following the specific method(s) and complying with set specifications a manufacturer can prove the safety of their products, however this does not mean it will automatically be approved. USP–NF is a combination of the United States Pharmacopeia (USP) and the National Formulary (NF). Monographs for drug substances, dosage forms, and compounded preparations are shown in the USP; monographs for dietary supplements and ingredients can be found in a separate section of the USP, and monographs for excipients can be found in the NF.

A generic drug (in plural generic drugs or generics) is a drug defined as "a drug product that is comparable to an ethical drug brand/reference listed drug product, considering dosage, quality and performance, and intended use. Generics just like ethical drugs must comply with the local regulations of the countries where they are distributed. Thus a generic drug must contain the same active ingredients as the original formulation, within an acceptable bioequivalent range with respect to pharmacokinetic and pharmacodynamic properties.

USP General Chapters

The United States Pharmacopeia (USP) – National Formulary (NF) is continuously revised, and the revisions are presented in twice-yearly supplements as standard revisions in the USP–NF.
The monographs highlighted in this compilation follow the USP37-NF32 (supplement 2), but are also compliant with the USP38-NF33 (active from May 1, 2015). More frequently, the revisions are published through different accelerated revision processes: Errata, Interim Revision Announcements (IRAs), Revision Bulletins, and Stage 6 Harmonization notices are posted on the USP website in the USP–NF section (http://www.usp.org/usp-nf).

In the general chapters you can find details about different tests and procedures referred to in multiple monographs, and in the general notices you find definitions for terms used in the monographs, as well as information that is necessary to interpret the monograph requirements are found. The following pages show some of these details that are relevant for the analytical techniques used within this compilation, namely chapters 621 (chromatography), and 711 (dissolution).

Dissolution Testing Workflow

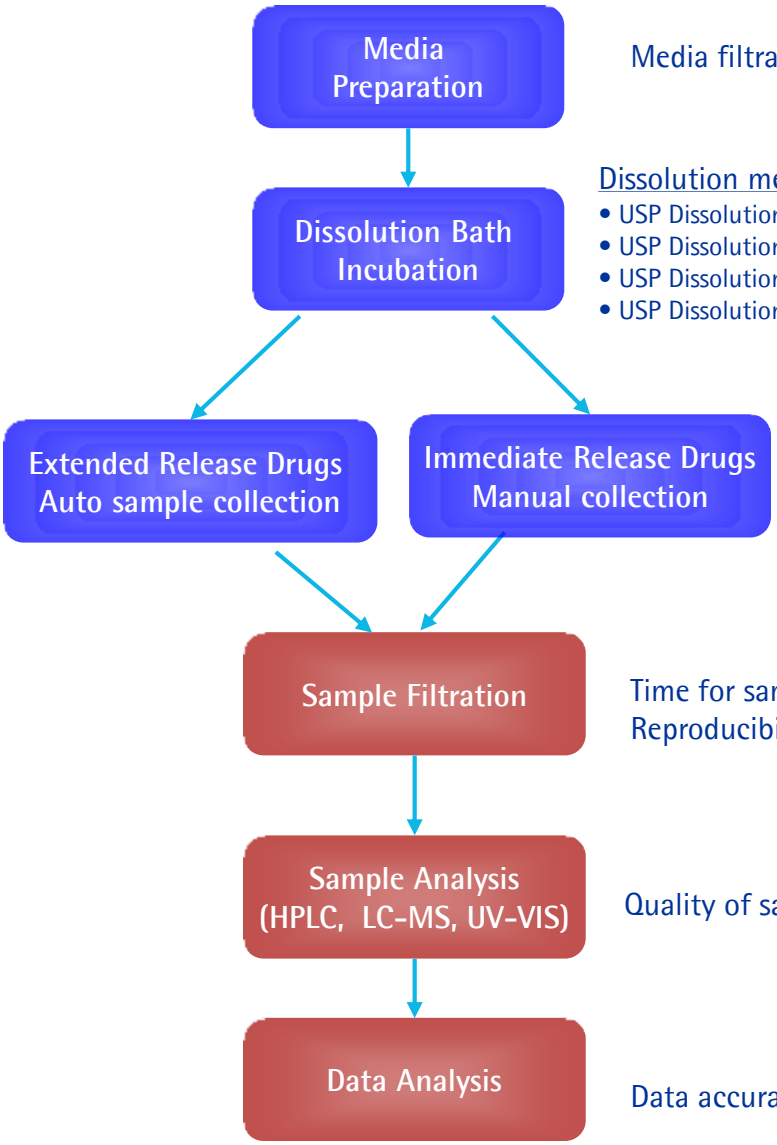
Various steps of drug dissolution work flow, along with some of the concerns at these various steps, are presented on the following page. Detailed information can be found in USP general chapter 711, which is also harmonized with the European Pharmacopeia and the Japanese Pharmacopeia. These three pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter.

In the general chapter 711, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified. The type of instrument used should follow the specification in the individual monograph. Where the label states that it is enteric-coated, and where a dissolution or disintegration test that does not specifically state that it is to be applied to delayed-release articles is included in the individual monograph, the procedure and interpretation given for Delayed-Release Dosage Forms is applied unless otherwise specified in the individual monograph. For hard or soft gelatin capsules and gelatin-coated tablets that do not conform to the Dissolution specification, repeat the test as follows. Where water or a medium with a pH of less than 6.8 is specified as the Medium in the individual monograph, the same Medium specified may be used with the addition of purified pepsin that results in an activity of 750,000 Units or less per 1000 mL. For media with a pH of 6.8 or greater, pancreatin can be added to produce not more than 1750 USP Units of protease activity per 1000 mL.

For oral dosage forms most commonly used dissolution apparatus is either Apparatus (App) 1 (Basket type) or App 2 (Paddle type), but other apparatus might be used for different dosage forms such as patch, suppository. Based on dosage form, often manual or automatic sample collection is selected, where the latter is more common for extended release formulations (preferred over manual collection). In all situations sample filtration is the key sample preparation step involved following sample collection.

Sample analysis is commonly carried out either using UV-VIS spectrophotometry or HPLC-UV. HPLC and UV-VIS spectroscopy are more or less used in equal proportions for sample analysis. LC-MS is very rarely used for analysis following dissolution. Spectrophotometry is used when the formulation is a single drug formulation with no interference from other components in the formulation. On the other hand, with multi-drug formulations or extended release formulations it is more likely that HPLC will be used for analysis. Finally data is analyzed to generate dissolution profile to compare it with the existing dissolution profile. The concerns that are listed on the following page are mainly associated with sample preparation and quality of components of media and mobile phase if HPLC analysis is carried out downstream.

Dissolution Testing Workflow



Media filtration and water quality

Dissolution method/apparatus:

- USP Dissolution Apparatus 1 - Basket (37°C)
- USP Dissolution Apparatus 2 - Paddle (37°C) – most common
- USP Dissolution Apparatus 3 - Reciprocating Cylinder (37°C)
- USP Dissolution Apparatus 4 - Flow-Through Cell (37°C)

Re-validation if switching from manual to automated system

Time for sample collection and preparation; Reproducibility, Instrument downtime

Quality of samples and mobile phase ingredients

Data accuracy and reproducibility

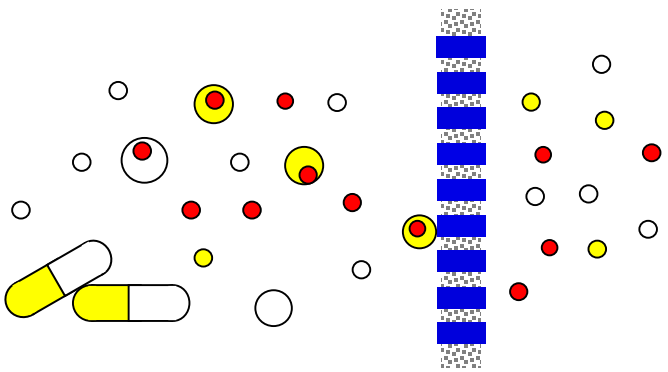
Media Preparation: De-aeration of the dissolution medium is a key concern here as presence of air / oxygen dissolved in dissolution medium affects dissolution profile. A well known example is Prednisone tablet which is a USP calibrator tablet used for calibrating dissolution baths and presence of oxygen in dissolution medium can affect rate of its dissolution.
Vacuum filtration is one of the well known methods of de-aeration of dissolution medium.

Why is Filtration Important in Drug Dissolution Testing?

Filtration stops the dissolution process

Dissolution media contains dissolved and undissolved inert ingredients and active compound.

...filtration removes undissolved ingredients...
...thereby providing more representative samples



= *Carrier, excipient or inert ingredient*

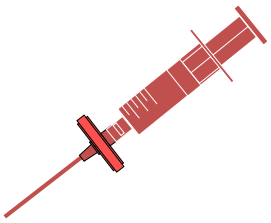
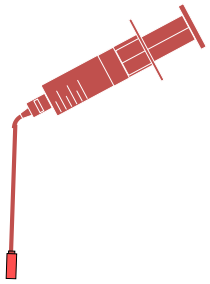
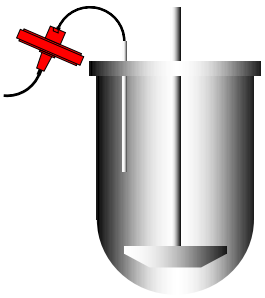
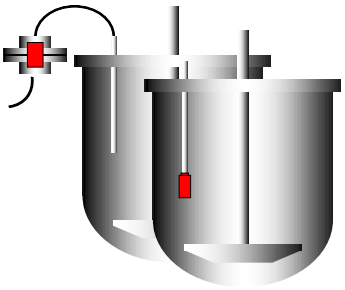
= *Drug or Active Compound*

- Dissolution medium contains dissolved and un-dissolved inert ingredient and active compound.
- Filtrate contains dissolved ingredients
  - A more representative sample of that time point.
- If solid particles are allowed to pass to the analysis step, inaccurate data can result either from particle interference or downstream dissolution of drug which was still un-dissolved.

Sample Preparation for Dissolution

A Typical USP Dissolution Protocol:

Determine amount of Quinidine Sulphate dissolved by UV absorption at 248 nm *on the* ***filtered*** *portion* of solution under test suitably diluted by the dissolution medium.

Sampling	Syringe Filters	Frits
Manual		
Automated		

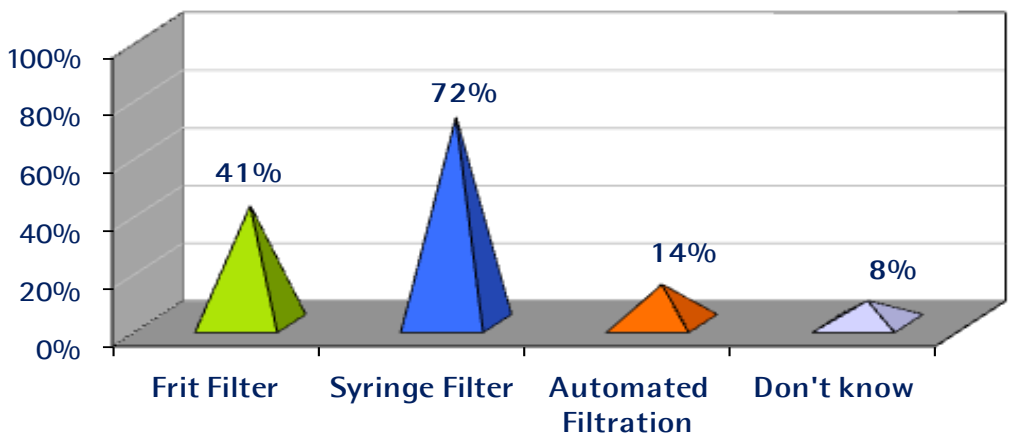
Filtration is a key element in dissolution testing and there are multiple ways of filtering samples following dissolution testing.

Frits / disks and syringe filters are the only two ways of sample preparation following dissolution and they can both be used in manual or automated formats.

Comparison of Filtration Method

	Syringe Filter	Filter Tips
Typical Pore Size (µm)	0.2, 0.45, 1.0	10 – 70
Pore Size Distribution	Narrow	Broad
Pore Size Rating	Largest Pore Size	Average
Material of Construction	PP, PTFE, PVDF, Nylon, PES	PE or PTFE
Back wash	Possible if membrane is wet	Yes
Reusable	No	Yes?
Automated / Manual Methods	Both	Both
Compatible with filter changing stations	Yes	No

Pore sizes for frits are typically large (10-70 µm), and commonly a 40-45 µm frit filter is used in drug dissolution testing. That means the sample needs to be further filtered using a syringe filter for downstream HPLC analysis. Even in UV analysis some interference can be observed due to presence of particulates. Inappropriate choice of sample preparation device can lead to problems in downstream analysis. It is therefore worth to keep in mind that a 30 or 40 µm frit can give similar filtration performance as the effective pore size is an average rather than largest pore. Important to remember is that frits are typically reused in a single dissolution run (in automated systems) whereas syringe filters will be discarded after a single filtration. Below is an overview of the relative use of filtration devices:



Syringe filters are used by > 80% of all dissolution labs

"Millipore Dissolution Market Survey, 2007"

Main Characteristics of Membranes Important for Sample Preparation

Pore Size and Porosity:

Pore size and porosity determine ability of syringe filters to retain particles. For formulations with micronized / nano particulate active, this information is critical for retention of the un-dissolved drug active.

Pore Symmetry:

Pore symmetry is defined by pore dimensions across the thickness of a membrane filter and it determines filtration flow rate. This is important parameter when filtering large volumes of dissolution medium.

Chemical Compatibility:

Knowledge about chemical compatibility of the membrane and the formulation under study ensures that membrane remains structurally integral during use. It also ensures that minimum amount of extractables are added to the sample during filtration.

Analyte Binding:

Binding characteristics of the membrane are dependant on physico-chemical characteristics of the membrane and the analyte under study. Excessive analyte binding leads to incorrect determination of drug dissolution. Usually drug binding can be reduced by washing of the membrane with sample before analysis.

Extractables:

Any surface that comes in contact with the sample has potential to add extractable impurities to the sample. Extractables are dependant on physico-chemical characteristics of the membrane as well as chemical compatibility of the membrane with dissolution medium under study. Similar to analyte binding, extractable impurities can also be reduced by washing the membrane with sample.

Drug Binding to Membrane Filters

Post dissolution testing, when samples are filtered using membrane filters, its important to take into account binding characteristics of membranes and the physic-chemical characteristics of analyte of interest.

Hydrophilic PTFE, nylon and hydrophilic PVDF membrane-based syringe filters are some of the most commonly used syringe filters in this application. Of the three membranes, nylon membrane shows the most drug binding tendency which can lead to erroneous results in dissolution testing.

The reasons for drug binding to nylon membrane are multifold:

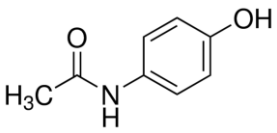
Nylon is a polyamide. It has an amino group at one end and a carboxylic acid group at the other end. Both these groups interact with analytes that are either basic or acidic in nature forming strong hydrogen bonds with analytes.

Depending on the pH of the dissolution medium, it can electrostatically interact with the analytes. Both these interactions together lead to drug binding behavior.

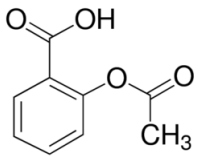
On the contrary, both hydrophilic PTFE and PVDF do not form these interactions with analytes thereby minimizing drug binding to these membranes. In order to reduce the effect of drug binding on quantitation, one method that researchers use is flushing the membrane filter with the sample prior to collecting sample for analysis. A typical flush with 3-5 ml of sample completely saturates the membrane surface thereby reducing drug binding significantly and providing quantitative analyte recovery. To exemplify this we have included an example, the monograph method for Excedrin tablets.

Acetaminophen, Aspirin and Caffeine (USP)

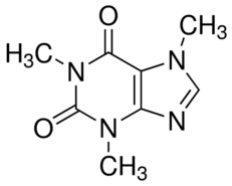
Excedrin Tablet Dissolution



Acetaminophen



Aspirin
(acetylsalicylic acid)



Caffeine

Excedrin is an over-the-counter headache pain reliever, often in the form of tablets or capsules. It contains acetaminophen (Tylenol), aspirin (acetylsalicylic acid), and caffeine. Presently Excedrin is produced by Novartis.

Drug dissolution testing has been carried out following the experimental conditions in the USP37-NF32 monograph for Acetaminophen, Aspirin, and Caffeine Tablets. Drug binding interactions were studied on Nylon, PTFE and PVDF filters. For Acetaminophen and Aspirin, substantial drug binding was observed in the first milliliter of filtrate, but not for caffeine.

For tablets exhibiting drug binding to the filters an initial flush with 3-5 ml of sample has been demonstrated to reduce drug binding and provide quantitative analyte recovery.

Acetaminophen, Aspirin and Caffeine (USP)

Excedrin Tablet Dissolution

Definition: Acetaminophen, Aspirin, and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ($C_8H_9NO_2$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$).

Identification

The relative retention times of the major peaks in the chromatogram of the Assay preparation correspond to those in the chromatogram of the Standard preparation, as obtained in the Assay.

Assay: HPLC

Mobile phase: Prepare a suitable mixture of water, methanol, and glacial acetic acid (69:28:3). Make adjustments if necessary (see System Suitability under chapter 621).

Internal standard solution: Prepare a solution of benzoic acid in methanol containing about 6 mg per mL.
Solvent mixture: Prepare a mixture of methanol and glacial acetic acid (95:5).
Standard stock solution: Dissolve accurately weighed quantities of USP Acetaminophen RS, USP Aspirin RS, and USP Caffeine RS in Solvent mixture to obtain a solution having known concentrations of about 0.25 mg of USP Acetaminophen RS per mL, 0.25J mg of USP Aspirin RS per mL, and 0.25J¢ mg of USP Caffeine RS per mL, J being the ratio of the labeled amount, in mg, of aspirin to the labeled amount, in mg, of acetaminophen per Tablet; and J¢ being the ratio of the labeled amount, in mg, of caffeine to the labeled amount, in mg, of acetaminophen per Tablet.

Standard preparation: Transfer 20.0 mL of Standard stock solution and 3.0 mL of Internal standard solution to a 50mL volumetric flask, dilute with Solvent mixture to volume, and mix. This solution contains about 0.1 mg of USP Acetaminophen RS, 0.1 mg of USP Aspirin RS, and 0.1 mg of USP Caffeine RS per mL.

Assay preparation: Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of acetaminophen, to a 100-mL volumetric flask. Add about 75 mL of Solvent mixture, and shake by mechanical means for 30 minutes. Dilute with Solvent mixture to volume, and mix. Transfer 2.0 mL of this solution and 3.0 mL of Internal standard solution to a 50-mL volumetric flask, dilute with Solvent mixture to volume, and mix.

Chromatographic system: (See Chromatography 621, System Suitability.)
Detector: UV 275 nm
Column: 100x4.6 mm column; 5 µm packing L1.
Column temperature: 45±1 °C
Flow rate: About 2 mL/min
Injection size: About 10 µL

Acetaminophen, Aspirin and Caffeine (USP)

Excedrin Tablet Dissolution

Chromatograph the Standard preparation, and record the responses as directed for the Procedure. The tailing factor for each analyte peak is not more than 1.2. The resolution, R, between any of the analyte and internal standard peaks is not less than 1.4 and the relative 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. The relative retention times are about 0.3 for acetaminophen, 0.5 for caffeine, 0.8 for aspirin, 1.0 for benzoic acid, and 1.2 for salicylic acid.
Calculate the quantities, in mg, of acetaminophen ($C_8H_9NO_2$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken by the formula: 2500C(RU / RS)

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the Standard preparation; and RU and RS are the ratios of the peak responses of the corresponding analyte and internal standard peaks of the Assay preparation and the Standard preparation, respectively.

This is an isocratic method and thus possible to scale and we used a Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column.

Dissolution <711>

HPLC

Medium: water; 900 mL.
Apparatus 2: 100 rpm.
Time: 60 minutes.

Mobile phase, Internal standard solution, Solvent mixture, Standard stock solution, and Chromatographic system—Proceed as directed in the Assay.

Standard preparation: transfer 20.0 mL of Standard stock solution, 3.0 mL of Internal standard solution, and 20 mL of water to a 50 mL volumetric flask, mix, and allow to stand for about 30 seconds. Dilute with solvent mixture to volume, and mix. Use within 8 hours.

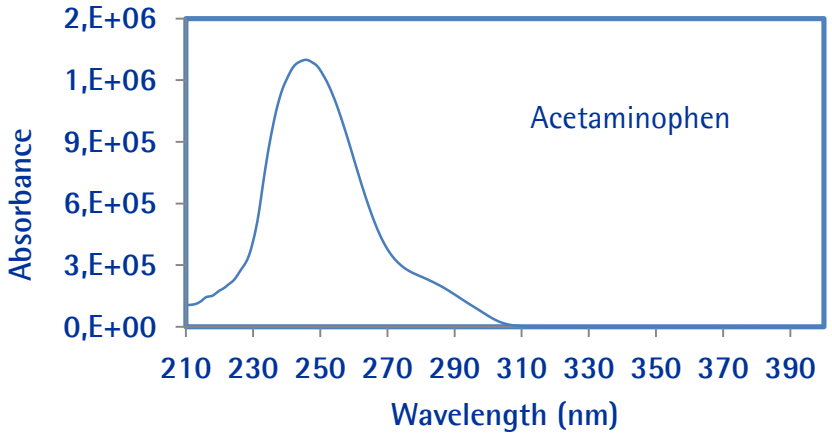
Test preparation: transfer 20.0 mL of a filtered portion of the solution under test to a 50-mL volumetric flask, add 3.0 mL of Internal standard solution and 20 mL of Solvent mixture, mix, and allow to stand for 30 seconds. Dilute with Solvent mixture to volume, and mix.

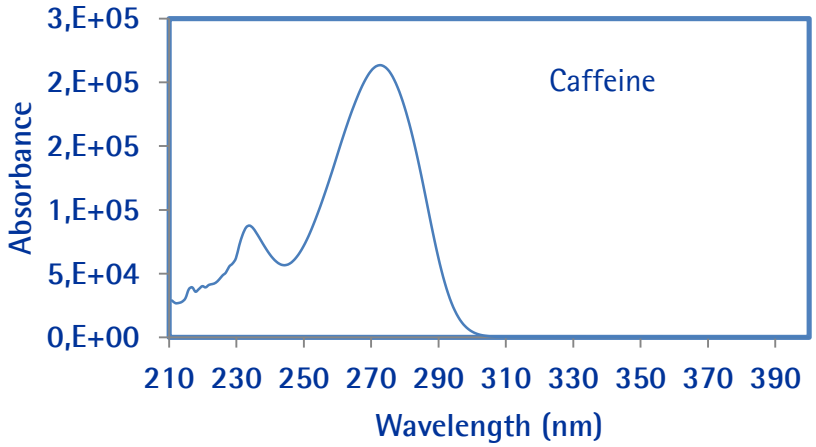
Procedure. Proceed as directed for Procedure in the Assay.

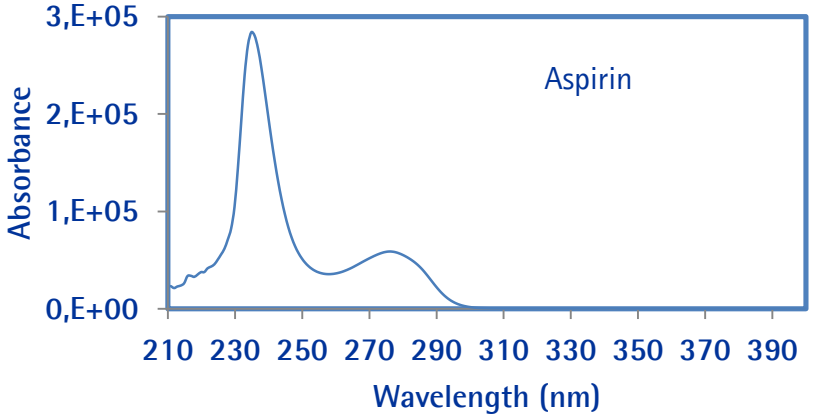
Not less than 75% (Q) of the labeled amounts of $C_8H_9NO_2$, $C_9H_8O_4$, and $C_8H_{10}N_4O_2$ is dissolved in 60 min.

Acetaminophen, Aspirin and Caffeine (USP)

Excedrin Tablet Dissolution





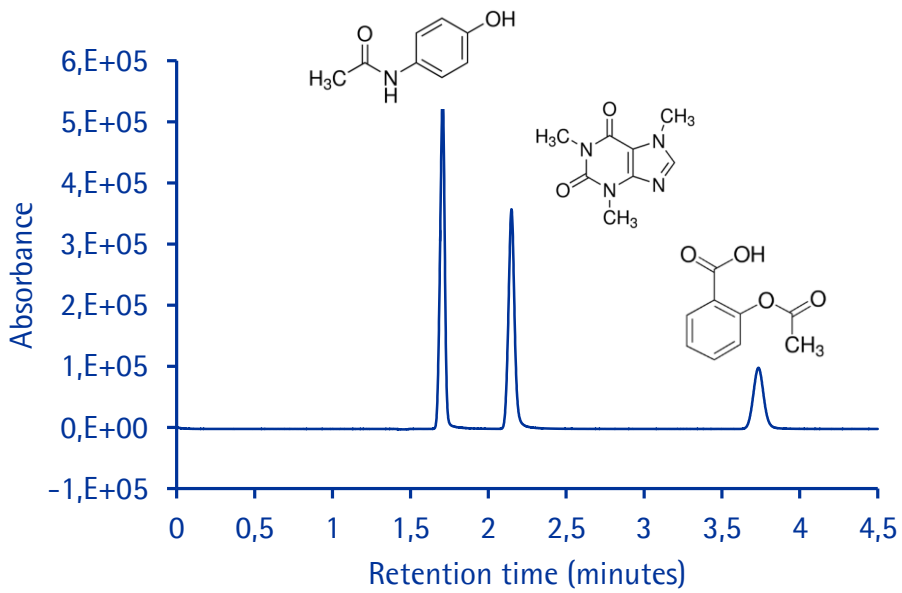


Acetaminophen, Aspirin and Caffeine (USP)

Excedrin Tablet Dissolution

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	5 µL	
Detection:	UV, 275 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Medium:	Water, 900 ml	
Apparatus 2:	100 rpm	
Mobile phase:	Mixture of water, acetonitrile and glacial acetic acid (69: 28: 3 v/v)	
Temperature:	45°C	
Solvent Mixture:	Prepare a solution of methanol and glacial acetic acid in (95: 5 v/v)o	
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:	Filter the sample after the dissolution is completed and inject as sample.	
Pressure Drop:		

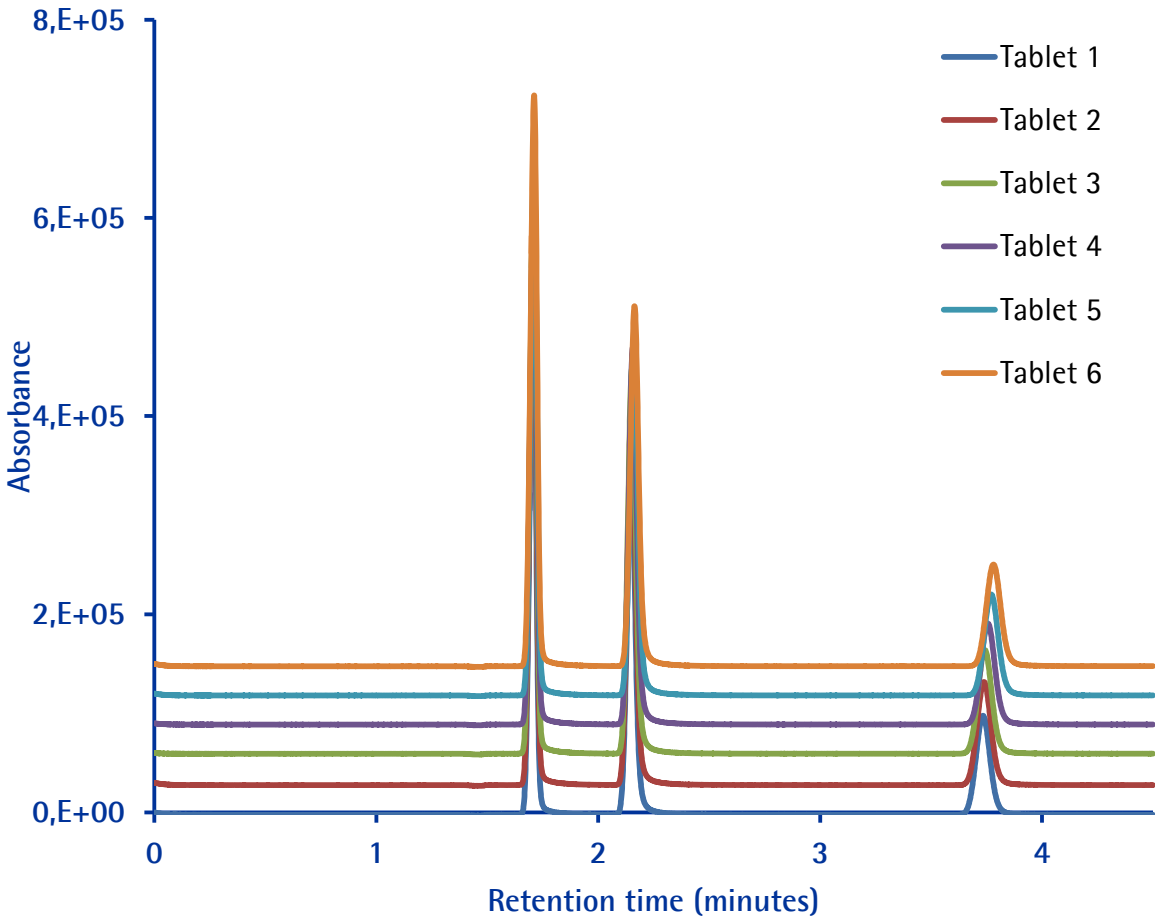


Chromatographic Data: Standard

No.	Compound	Retention Time (min)	Tailing factor	Resolution
1	Acetaminophen	1.7	0.97	-
2	Caffeine	2.2	1.03	7.1
3	Aspirin	3.7	1.04	16.8

Acetaminophen, Aspirin and Caffeine (USP)

Excedrin Tablet Dissolution



Recovery after dissolution for the three molecules in Excedrin tablets were determined using the following equation:

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

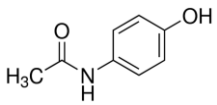
rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of the Standard solution (mg/mL)
L = label claim (mg/Capsule)
V = volume of Medium, 900 mL

Acetaminophen, Aspirin and Caffeine (USP)

Excedrin Tablet Dissolution

Filtrate Used	Syringe Filter Used		
	Millex PTFE	Millex Nylon	Millex PVDF
1st ml	110.4	58.4	109.0
2nd ml	112.3	109.3	110.6
3rd ml	112.2	111.3	110.8
5th ml	112.2	111.5	110.7

Acetaminophen Recovery (%):

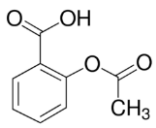


Drug binding of acetaminophen observed for 1st ml of filtrate with Nylon syringe filters.
No drug binding was observed for Millex PTFE and Millex PVDF syringe filters

Tolerance: NLT (not less than) > 75% of the labeled amount of Acetaminophen

Filtrate Used	Syringe Filter Used		
	Millex PTFE	Millex Nylon	Millex PVDF
1st ml	119.5	28.2	117.4
2nd ml	121.4	116.1	119.0
3rd ml	121.2	121.6	119.1
5th ml	121.3	122.0	119.2

Aspirin Recovery (%):

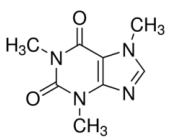


Drug binding of Aspirin observed for 1st ml of filtrate when using Nylon syringe filters.
No drug binding was observed for Millex PTFE and Millex PVDF syringe filters

Tolerance: NLT > 75% of the labeled amount of Aspirin dissolved.

Filtrate Used	Syringe Filter Used		
	Millex PTFE	Millex Nylon	Millex PVDF
1st ml	114.2	106.0	112.2
2nd ml	113.9	112.6	112.6
3rd ml	113.8	112.9	112.7
5th ml	113.6	112.9	112.7

Caffeine Recovery (%):



No drug binding was observed for Nylon, Millex PTFE or Millex PVDF syringe filters

Tolerance: NLT > 75% of the labeled amount of Caffeine hydrochloride is dissolved.

Improving a Method is Not Only About Speed – It's Also About Sensitivity and Tolerance Against Sample Matrix!

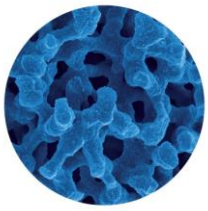
At present in liquid chromatography it is possible to reach extremely fast flow velocities, and with high separation efficiencies, using particle packed UHPLC or solid-core particle columns but often with substantial downtimes during washing and re-equilibration of the column due to sample matrix issues. In dissolution testing samples are only filtered and thus considerably more "dirty" than standard solution samples and/or samples that has gone through sample clean-up before the HPLC analysis.

What if you could shorten the overall analysis time by 50% or more?

The new Chromolith® HighResolution monolithic silica columns offer a major advantage of providing high separation efficiency yet at low column back pressure, allowing you to vary the flow rate over a large range up to very high linear flow velocities even when you analyze a more "dirty" sample. Thus if you have sufficient chromatographic space ,i.e. resolution between your peaks, you can easily increase the flow rate (you can even use flow gradients) and save time.
On pages 17-24 you can find out how to scale a monograph method within allowable limits.

Chromolith® HighResolution columns provide:

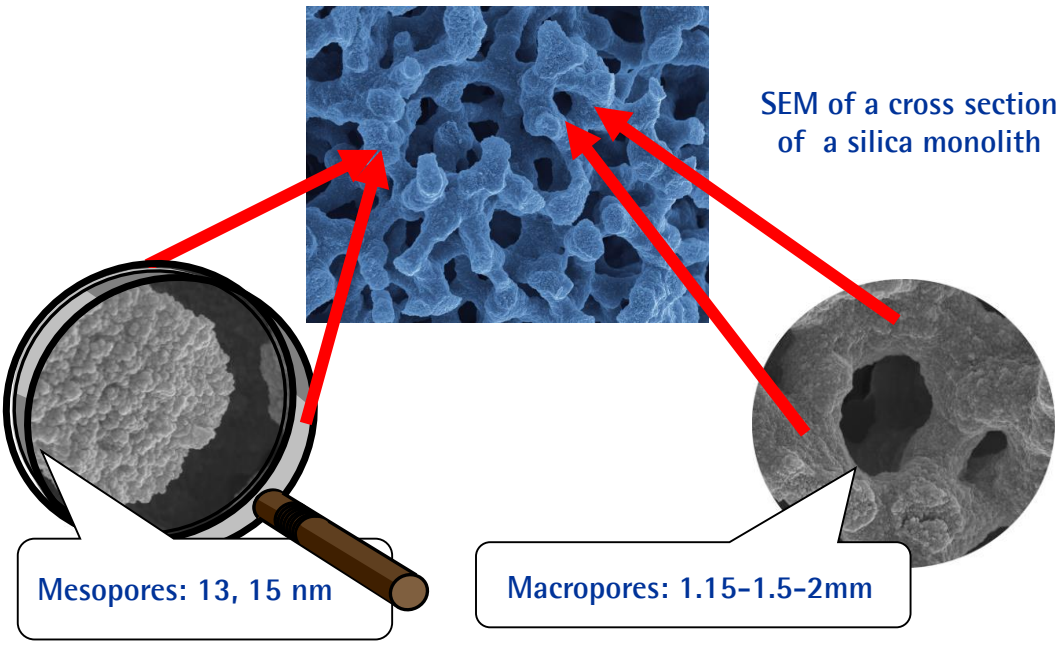
- High chromatographic efficiency and wide flow rate range that allow faster analysis
  - Ultra high performance separation without ultra-high column pressure
- Improved peak shape/symmetry
- Prolonged column lifetime
- Reduced column back pressure
- Can be used on any standard HPLC or UHPLC equipment
- Based on unique and patented monolithic silica technology



SEM picture of a cross selection from a silica monolith: Total porosity > 80%

Monolithic silica Columns
- With rigid bimodal pore structure

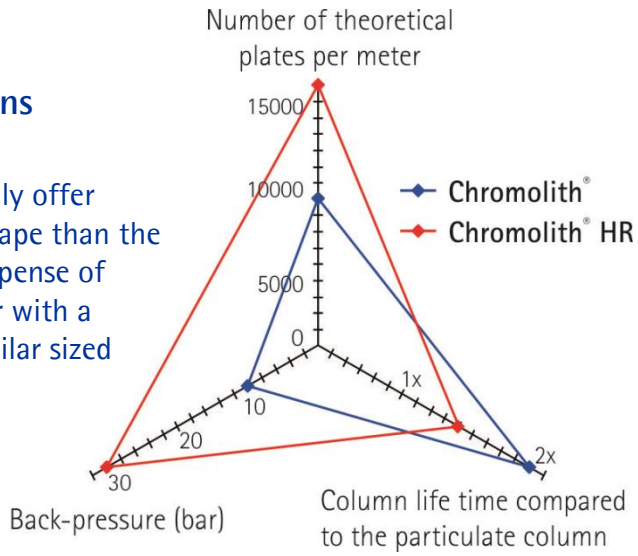
Chemically the same as high purity silica particles



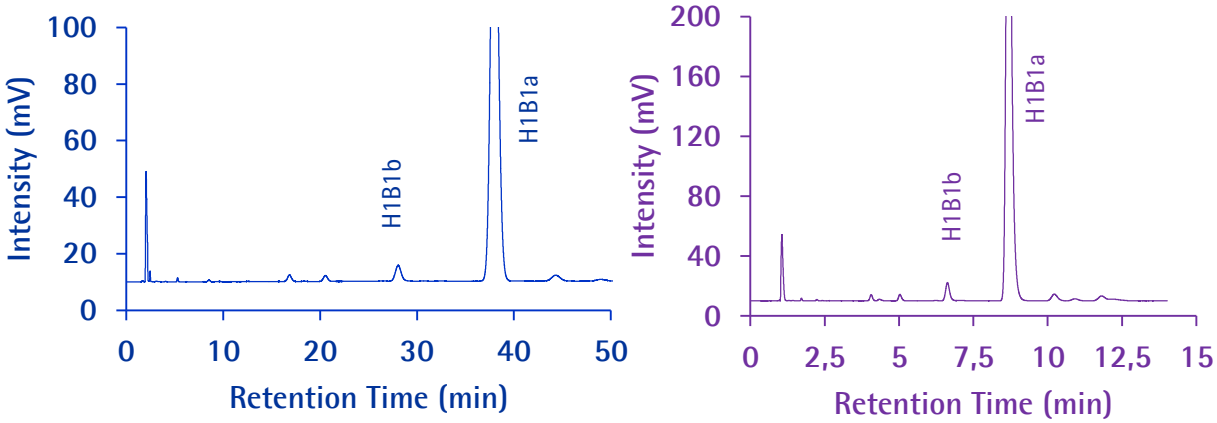
Chromolith® vs Chromolith® HighResolution

Chromolith® HighResolution
– the faster way to trouble-free separations

The new Chromolith® HighResolution column family offer higher separation efficiency and improved peak shape than the first generation Chromolith® column but at the expense of slightly higher column back-pressure, still however with a two-fold lower back pressure compared with a similar sized particulate packed column.



Advantages with Monolithic Columns



Columns:	Analytical column RP-18 250x4.6 mm, 5 µm	
	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	
Injection:	20 µL	
	10 µL	
Detection:	UV 245 nm (standard HPLC system used with both columns)	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Acetonitrile:Methanol:Water 106:55:39 (v/v/v)	
Temperature:	Ambient	
Diluent	Methanol	
Sample:	0.4 mg / ml in diluent	
Pressure Drop:		**118 Bar (1711 psi)**
		50 Bar (725 psi)

Parameter	Old Method	New Method	Savings
Runtime	60 min	15 min	**45 min/analysis**
Solvent usage	60x1.5 = 90 ml	15x1.5 = 22.5 mL	**67.5 mL/analysis**
Total analysis/day	8 samples	32 samples	**4-fold productivity increase**

Due to better peak symmetry in Chromolith® HighResolution columns it is very easy to transfer monograph methods (Pharmacopoeial methods) without changing any major parameter, as visualized below with the Ivermectin USP method transfer

USP Chapter 621 – Chromatography

What changes are allowed in a monograph method?

- Can we change the column material?
- Are we allowed to use a different column dimension?
- Is it allowed to scale down to smaller ID columns to save solvent?
- Is there a possibility to speed up separation?

The answer is "yes" to all these questions...but how?

Factors that may affect chromatographic behavior:

1. Composition, ionic strength, temperature, and apparent pH of the mobile phase

2. Flow rate, column dimensions, column temperature, and pressure

3. Stationary phase characteristics, including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, and specific surface area

4. Reversed-phase and other surface modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading, etc.)

In some circumstances, it may be desirable to use an HPLC column with different dimensions to those prescribed in the official procedure (different length, internal diameter, and/or particle size). Adjustments to the physical dimensions are allowed to a certain extent, defined within the guidelines of the pharmacopeia, outlined on pages 27 and 28. However, changes in the chemical characteristics ("L" designation) of the stationary phase are considered to be a modification to the method and require full validation. Adjustments to the composition of the mobile phase in gradient elution may cause changes in selectivity and are not recommended. If adjustments are necessary, change in column packing (maintaining the same chemistry), the duration of an initial isocratic hold (when prescribed), and/or dwell volume adjustments are allowed. Additional allowances for gradient adjustment are noted in the following text and table for USP monographs.

If you need guidance or suggestions with your analytical chromatography needs, please send an email to chromatography@emdmillipore.com

USP Packings (L classifications)
EMD Millipore Columns

Packing	Description	Chemistry
L1	Octadecylsilane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, **or a monolithic rod.**	RP-18 (C_{18} or ODS)
L3	Porous silica particles, 1.5 - 10 µm in diameter**, or a monolithic rod.**	Silica (Si)
L7	Octylsilane chemically bonded to totally porous or superficially porous silica particles 1.5 to 10 µm in diameter, **or a monolithic rod.**	RP-8 (C_8)
L9	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10 µm in diameter, **or a monolithic rod.**	NH2
L10	Nitrile groups chemically bonded to porous silica particles 1.5 to 10 µm in diameter , **or a monolithic rod.**	CN
L11	Phenyl groups chemically bonded to porous silica particles 1.5 to 10 µm in diameter, **or a monolithic rod.**	Phenyl
L20	Dihydropropane groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter, **or a monolithic rod.**	Diol
L29	Gamma alumina, reverse-phase, low carbon percentage by weight, alumina-based polybutadiene spherical particles, 5 µm in diameter with a pore volume of 80 angstrom units.	Alumina
L45	Beta cyclodextrin, R,S-hydroxypropyl ether derivative, bonded to porous silica particles, 5 - 10 µm in diameter.	Cyclodextrin *(Chiral)*

As of November 2014 the USP has extended the general description to include...."or a monolithic rod"..to the L9, L10, L11 and L20 packings definition.
This update is offical and published in the pharmacopeial forum (PF).

	USP	EP
Column Length**	See next page. NEW in USP 37!	± 70%
Column I.D.	See next page. NEW in USP 37!	± 25%
Particle Size	See next page. NEW in USP 37!	Reduction of 50%, no increase
Flow rate	See next page. NEW in USP 37!	± 50%
Column Temp.	± 10° C	± 10° C (max 60° C)
Injection Volume	can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits. Note that excessive injection volume can lead to unacceptable band broadening, causing a reduction in N and resolution. Applies to both gradient and isocratic separations	May be decreased (if LOD and repeatability is OK)
pH	± 0.2 units (both isocratic and gradient)	± 0.2 units
UV Wavelength	No adjustment is permitted	No adjustment is permitted
Buffer salts concentration	± 10% if the permitted pH variation is met.	± 10%
Mobile phase Composition	± 30% relative, or ± 10% absolute whichever is smaller	± 30% relative, or ± 30% absolute whichever is larger

** A guard column may be used with the following requirements, unless otherwise is indicated in the individual monograph (USP):

(a) the length of the guard column must be NMT (not more than) 15% of the length of the analytical column,
(b) the inner diameter must be the same or smaller than that of the analytical column, and
(c) the packing material should be the same as the analytical column (e.g., silica) and contain the same bonded phase.

(b) In any case, all system suitability requirements specified in the official procedure must be met with the guard column installed.

Particle Size (HPLC):
For isocratic separations, the particle size and/or the length of the column may be modified provided that the ratio of the column length (L) to the particle size (dp) remains constant or into the range between −25% to +50% of the prescribed L/dp ratio. Alternatively (as for the application of particle-size adjustment to superficially porous particles), other combinations of L and dp can be used provided that the number of theoretical plates (N) is within −25% to +50%, relative to the prescribed column.

Caution should be taken when the adjustment results in a higher number of theoretical plates which generates smaller peak volumes, which may require adjustments to minimize extra-column band broadening by factors as instrument plumbing, detector cell volume and sampling rate, and injection volume. **When particle size is not mentioned in the monograph, the ratio must be calculated using the largest particle size consigned in the USP definition of the column. For gradient separations, changes in length, column inner diameter and particle size are not allowed.**

Flow Rate (HPLC):
When the particle size is changed, the flow rate may require adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height). Flow rate changes for both a change in column diameter and particle size can be made by:

$$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$$

where F_1 and F_2 are the flow rates for the original and modified conditions, respectively; dc_1 and dc_2 are the respective column diameters; and dp_1 and dp_2 are the particle sizes. When a change is made from ≥3 µm to <3 µm particles in isocratic separations, an additional increase in linear velocity (by adjusting flow rate) may be justified, provided that the column efficiency does not drop by more than 20%. Similarly, a change from <3 µm to ≥ 3 µm particles may require additional reduction of linear velocity (flow rate) to avoid reduction in column efficiency by more than 20%.

Changes in F, dc, and dp are not allowed for gradient separations.
Additionally, the flow rate can be adjusted by ±50% (isocratic only).

EXAMPLES: Adjustments in column length, internal diameter, particle size, and flow rate can be used in combination to give equivalent conditions (same N), but with differences in pressure and run time. The following table lists some of the more popular column configurations to give equivalent efficiency (N), by adjusting these variables.

Changes in USP37

Length (L, mm)	Column Diameter (dc, mm)	Particle Size (dp, µm)	Relative Values				
			L/dp	F	N	Pressure	Run Time
250	4.6	10	25000	0.5	0.8	0.2	3.3
150	4.6	5	30000	1.0	1.0	1.0	1.0
150	2.1	5	30000	0.2	1.0	1.0	1.0
100	4.6	3.5	28600	1.4	1.0	1.9	0.5
100	2.1	3.5	28600	0.3	1.0	1.9	0.5
75	4.6	2.5	30000	2.0	1.0	4.0	0.3
75	2.1	2.5	30000	0.4	1.0	4.0	0.3
50	4.6	1.7	29400	2.9	1.0	8.5	0.1
50	2.1	1.7	29400	0.6	1.0	8.5	0.1

For example, if a monograph specifies a 150×4.6 mm; 5 µm column operated at 1.5 mL/min, the same separation may be expected with a 75×2.1mm; 2.5 µm column operated at 1.5 mL/min × 0.4 = 0.6 mL/min, along with a pressure increase of about four times and a reduction in run time to about 30% of the original.

Injection Volume (HPLC):
The injection volume can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits. Note that excessive injection volume can lead to unacceptable band broadening, causing a reduction in N and resolution. Applies to both gradient and isocratic separations.

The easiest approach to scale the injection volume is to compare differences in column tube volume and to keep same volumetric ratio between tube volume and injection volume, and thereby same volume loading on the column. A method scaled from a 250x4.6 to 100x2.1 mm column require a 12-fold reduction of injection volume using simple volume calculation of a tube (i.e. 250x4.6 =4.15 mL and 100x2.1 =0.346 mL). Thus if injection volume is 20 µL on the larger column, it is recommended to inject not more than 2 (1.7) µL on the smaller column.

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 are 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

Deviation is not permitted from the specified wavelength. 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.

Choosing the right Column to meet Monograph Specifications

The HPLC column choice is a very important consideration or it will be difficult to meet the set requirements in a monograph method. In the chapter discussing column selection, we have outlined which USP classification (code) our HPLC columns belong to.
At present, EMD Millipore offers L1, L3, L7, L8, L10, L11, L20, L29 and L45 modifications.

In addition, the USP has a database for chromatography columns to help users cross-reference HPLC columns. However, it is important to keep in mind that this database is only a tool as *"the database itself is not part of the text of USP–NF, and does not constitute an official interpretation of such text. The databases being displayed at the site are provided for informational purposes only to assist users in finding HPLC columns equivalent to that used to develop and validate a particular chromatographic procedure. After finding suggestions of equivalent columns using the databases, the columns should be tested with the appropriate sample. USP and the authors of the databases are not responsible for the results obtained with the columns proposed by the databases and such results should not be relied on to demonstrate compliance with USP standards or requirements. The data being provided by the databases were generated using brand new columns. USP has no information on and disclaims any knowledge of how these procedures will perform when evaluating already used columns".*

We at EMD Millipore believe that our columns can easily meet monograph specifications despite the fact that they may seem very different from the column used when developing the original monograph method. It is important to keep in mind that specific columns mentioned in USP as monograph columns are only examples –the monograph defines and prescribes only the column geometry and chemistry ("L" classification) which must be used. The user is free in the choice of column brand.

System Suitability Test (SST)
To verify and validate a monograph method and meet set requirements defined, system suitability tests are described.

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

As long as the changes of a monograph method are within the limits shown above it is possible to carry out only a partial revalidation followed by internal documentation of the updated method. If the changes are beyond limits, a complete revalidation and documentation is required followed by a discussion with an auditor and regulating authorities for approval of the new method. It is (of course) also possible to submit completely new monograph methods to authorities.

Validation and Verification

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

Validation of Compendial Procedure <1225>
Defines analytical performance characteristics
1. Recommends data for submission to USP-NF
2. Provides guidance on which analytical performance characteristics are needed based on the type of test
3. Incorporates ICH guidelines Q2A and Q2B

Performance Characteristics	Category 1	Category 2		Category 3	Category 4
		Quantitative	Limit Test		
Accuracy	Yes	Yes	-	-	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	-	Yes
LOD	No	Yes	Yes	-	No
LOQ	No	No	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.

Verification consists of assessing selected Analytical Performance Characteristics, such as those described in chapter 1225, to generate appropriate, relevant data rather than repeating the validation process. The table below illustrates required tests for the USP chapters dealing with validation and verification.

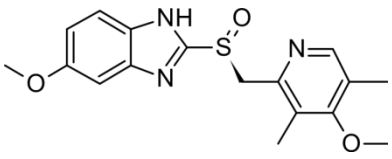
Performance	Validation	Verification
Accuracy	Yes	No
Precision	Yes	Maybe
Specificity	Yes	Yes
LOD	No	No
LOQ	Yes	Yes
Linearity	Yes	No
Range	Yes	No

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.

Esomeprazole Magnesium (USP)

– Delayed Release Capsules



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.
Patent expired in 2014

Drug dissolution testing has been carried out following the experimental conditions in the USP37-NF32 monograph for Esomeprazole magnesium delayed release capsules (*using an isocratic HPLC method with RP-18 endcapped columns and thus scalable*).

We have transferred this method to a monolithic column. The new method turned out to be three times faster, having improved chromatographic resolution, lower column backpressure, and still meeting all method performance criteria compared to the prescribed column. Clearly an added value!

In addition, the powdered pellets (about 80–90 mg), from the capsule content was also analyzed according to the method for related impurities (*a gradient method and of that reason not possible to scale to any other column than the prescribed column*). The presented data comply with all method performance criteria.

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 a pH 11.0 diluent as follows. 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.
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: 150x4.6 mm; 5 µm packing L1.
Flow rate: 1 mL/min
Injection size: 20 µL

This is an isocratic method and thus possible to scale.
We first used a Purospher® STAR RP-18 endcapped (5 µm) 150x4.6 mm column and later transferred the method to a Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column.

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: 100x4.6 mm column; 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. ($C_{17}H_{19}N_3O_4S$)

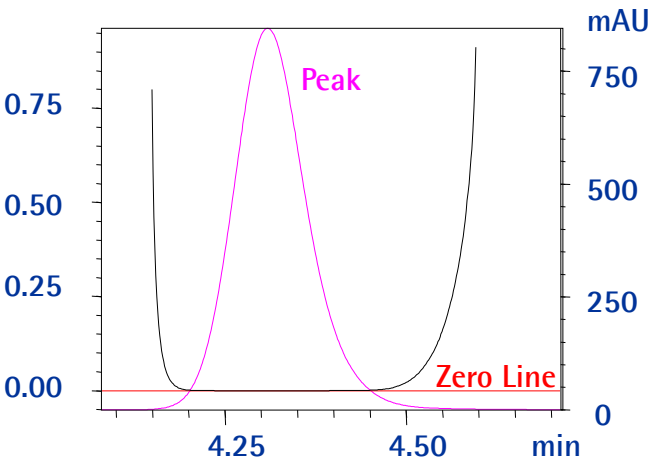
Esomeprazole Magnesium Capsules (USP)

Purospher® STAR RP-18 endcapped – Dissolution (Assay)

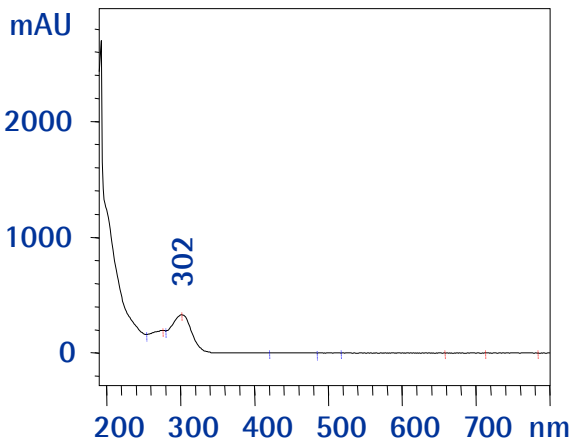
Chromatographic Conditions

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

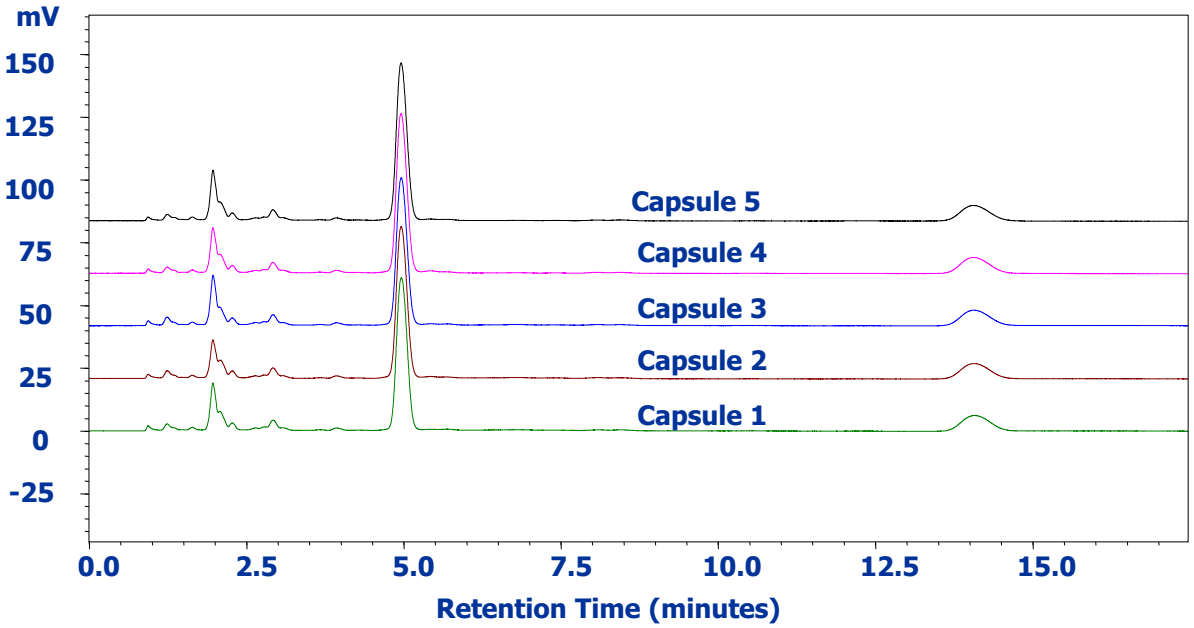


UV Analysis



Esomeprazole Magnesium Capsules (USP)

Purospher® STAR RP-18 endcapped – Dissolution (Assay)



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

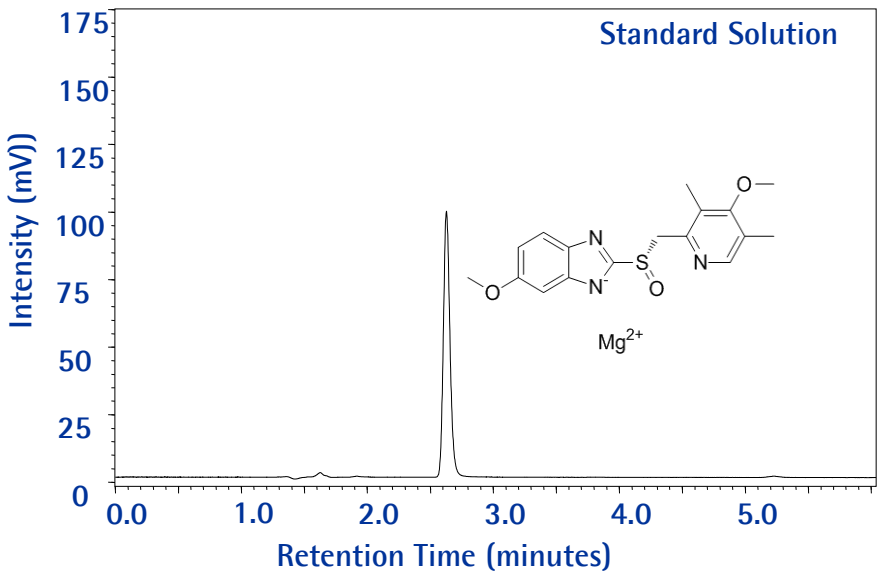
Acceptance criteria: NLT 75% of the claimed esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

Esomeprazole Magnesium Capsules (USP)

Chromolith® HR RP-18 endcapped - Dissolution (Assay)

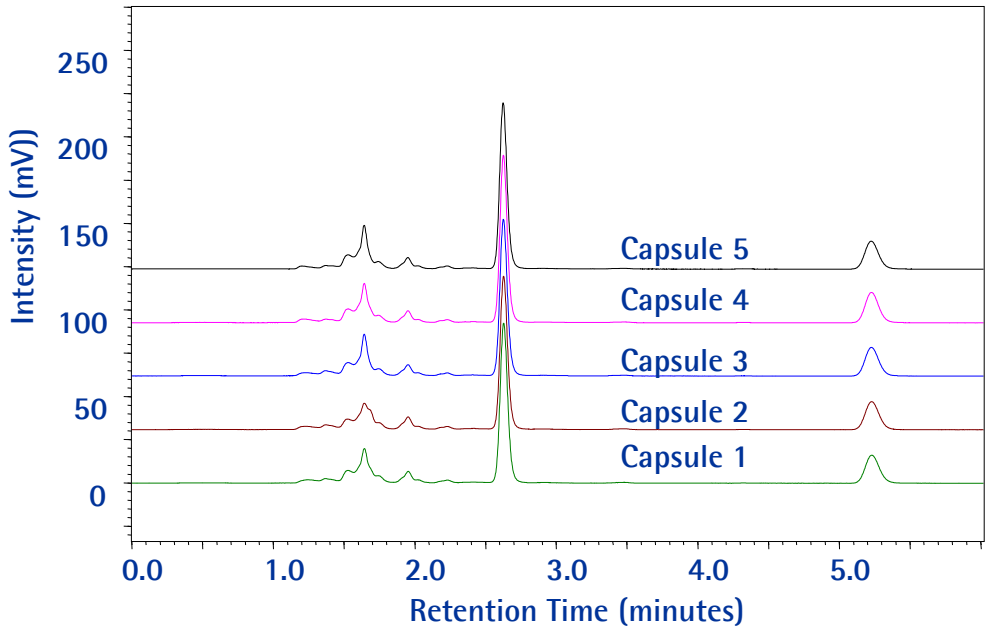
Chromatographic Conditions

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® HR RP-18 endcapped – Dissolution (Assay)



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.

Esomeprazole Magnesium Capsules (USP)

Purospher® STAR RP-18 endcapped – Related Impurities

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm 1.50469.0001

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

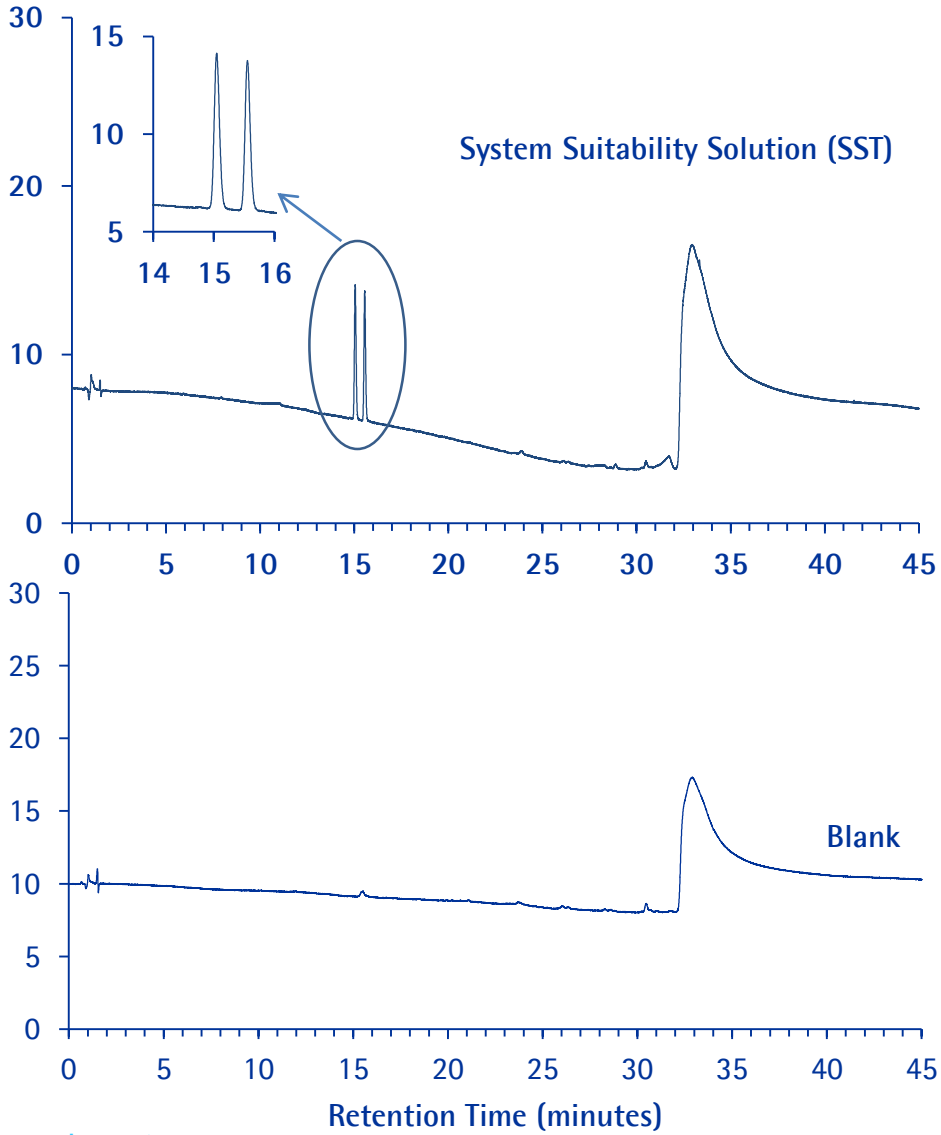
Standard Solution: 1 µg/mL each of USP Omeprazole and USP Omeprazole Related Compound A in methanol

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

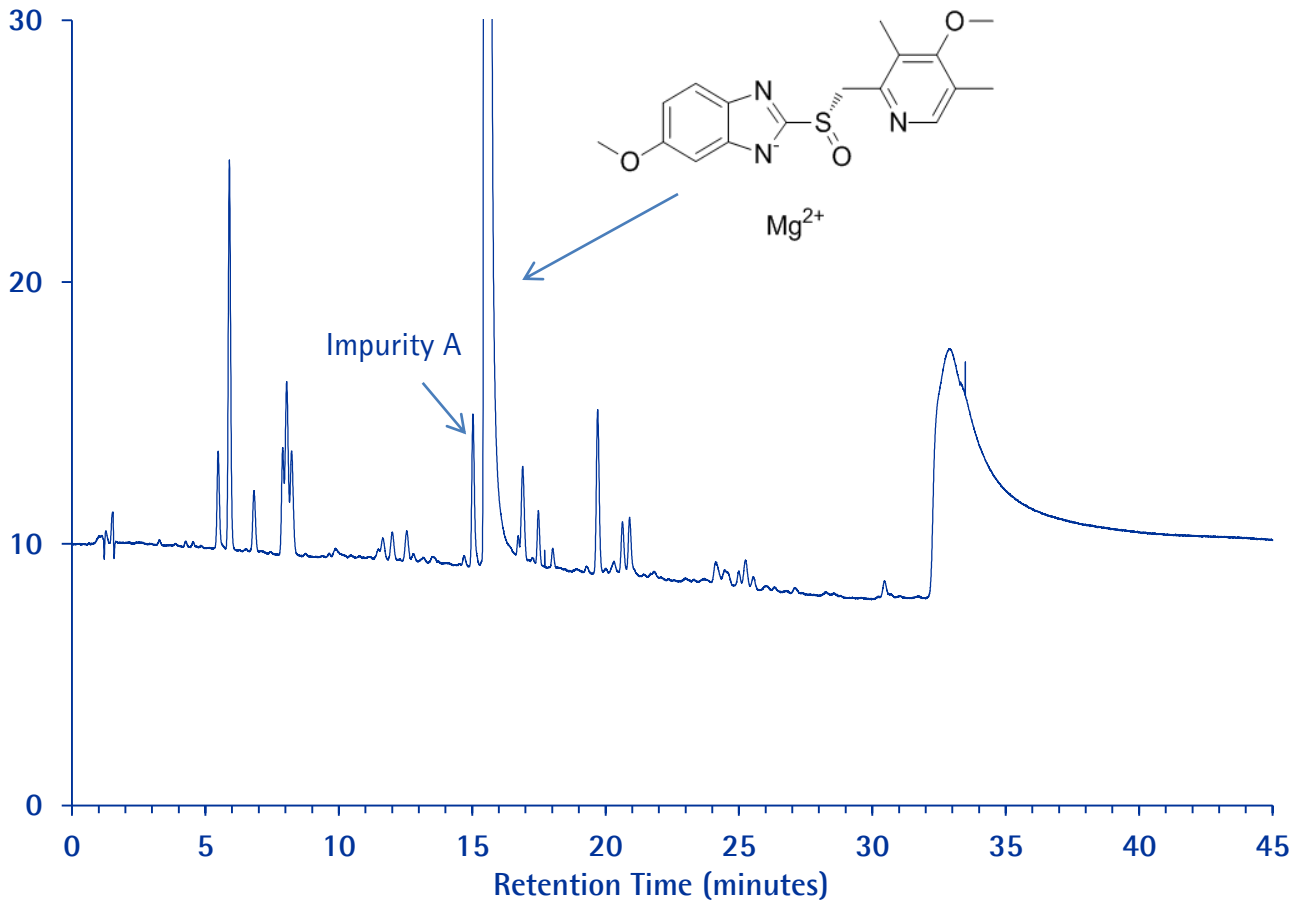
Chromatographic Data:

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)



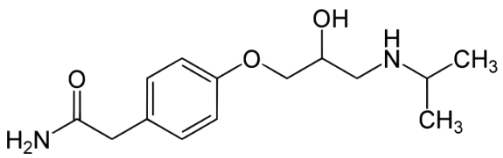
Suitability requirements
Resolution: NLT 2.5 between omeprazole related compound A and omeprazole
Relative retention time (RRT): 0.8 for and 1.0 for omeprazole related compound A and omeprazole, respectively

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

Atenolol (USP)

– Tablets



Atenolol is a selective β1-receptor antagonist, a beta blocker or β-blocker.
Atenolol was developed as a replacement for propranolol in the treatment of hypertension:

Drug dissolution of Atenolol Tablets has been carried out following the experimental conditions in the USP37-NF32 monograph for Atenolol Tablets (using an isocratic HPLC method with RP-18 endcapped columns and thus scalable). A 300x3.9 mm column is prescribed with L1 packing operating at 0.6 mL/min. To improve sample throughput we have transferred this method to a 100x4.6 mm long monolithic column.

The new method is much faster, provides improved chromatographic resolution and enhanced sensitivity, with lower column backpressure, and still meets all method performance criteria compared to the prescribed column geometry.

Atenolol (USP)

– Tablets

Dissolution <711> HPLC

Medium: 0.1 N acetate buffer, pH 4.6 (prepared by mixing 44.9 parts (v/v) of 0.1 N sodium acetate with 55.1 parts (v/v) of 0.1 N acetic acid solution, and adjust with either diluted sodium hydroxide or diluted acetic acid to a pH of 4.6); 900 mL
Apparatus 2: 50 rpm
Time: 30 min
Determine the amount of $C_{14}H_{22}N_2O_3$ dissolved by using the following method. Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the Atenolol Assay.
Standard solution: 0.01 mg/mL of USP Atenolol RS in Mobile phase
Sample solution: Pass a portion of the solution under test through a suitable 0.45 µm filter. Quantitatively dilute a measured volume of the filtrate with Mobile phase to obtain a solution estimated to contain about 0.01 mg/mL of atenolol.
Analysis: Proceed as directed in the Assay.
Calculate the percentage of $C_{14}H_{22}N_2O_3$ dissolved:

$$\text{Result} = (rU/rS) \times CS \times V \times D \times (100/L)$$

rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of USP Atenolol RS in the Standard solution (mg/mL)
V = volume of Medium, 900 mL
D = dilution factor for the Sample solution
L = Tablet label claim (mg)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{14}H_{22}N_2O_3$ is dissolved.

Atenolol Assay:
Mobile phase: 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, and pass through a filter having a 0.5 µm or finer porosity. Degas this solution before use.
Standard solution: 0.01 mg/mL of USP Atenolol RS in Mobile phase
Sample stock solution: Transfer 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of Mobile phase, and sonicate for 15 min to disintegrate the Tablets. Dilute with Mobile phase to volume.
Sample solution: Centrifuge a portion of the Sample stock solution, and dilute a volume of the supernatant with Mobile phase to obtain a solution nominally containing 0.01 mg/mL of atenolol.
Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 226 nm
Column: 300x3.9 mm column; packing L1
Flow rate: 0.6 mL/min
Injection size: 10 µL

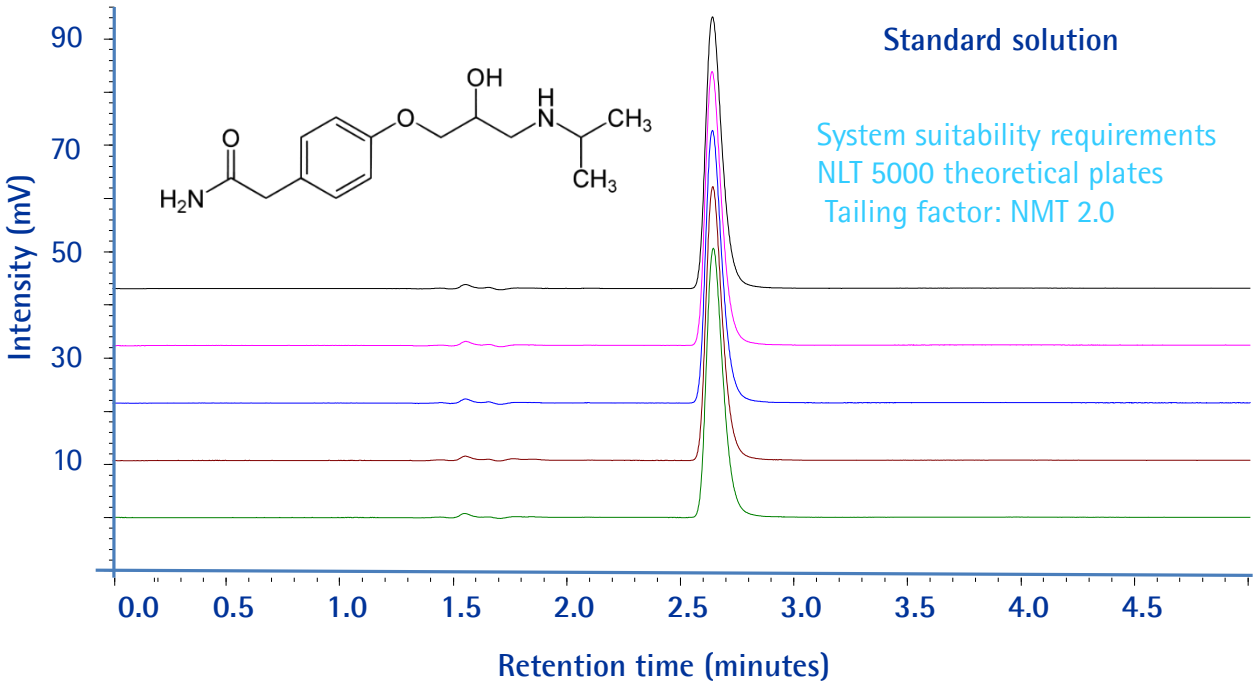
System suitability requirements
Column efficiency: NLT 5000 theoretical plates
Tailing factor: NMT 2.0

Atenolol Tablets (USP)

– Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	10 µl	
Detection:	UV 226 nm	
Cell:	10 µL	
Flow Rate:	1 mL/min	
Mobile Phase:	1.10 g of sodium 1 heptane sulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 ml of water. Add 2 ml of Dibutylamine and adjust pH = 3 with 0.8 M phosphoric acid. Add 300 ml Methanol and pass through 0.45µm filter.	
Temperature:	35°C	
Diluent :	0.1N acetate buffer pH= 4.6 (prepared by mixing 44.9 parts(v/v) of 0.1N Sodium acetate with 55.10 parts (v/v) of 0.1N acetic acid solution and adjust with either diluted sodium hydroxide or diluted acetic acid to a pH= 4.6)	
Sample solution:	Pass a portion of the solution under test through 0.45µm filter and inject.	
Standard solution :	0.01 mg/ml of USP Atenolol in mobile phase	
Pressure Drop:	92 Bar (1334 psi)	

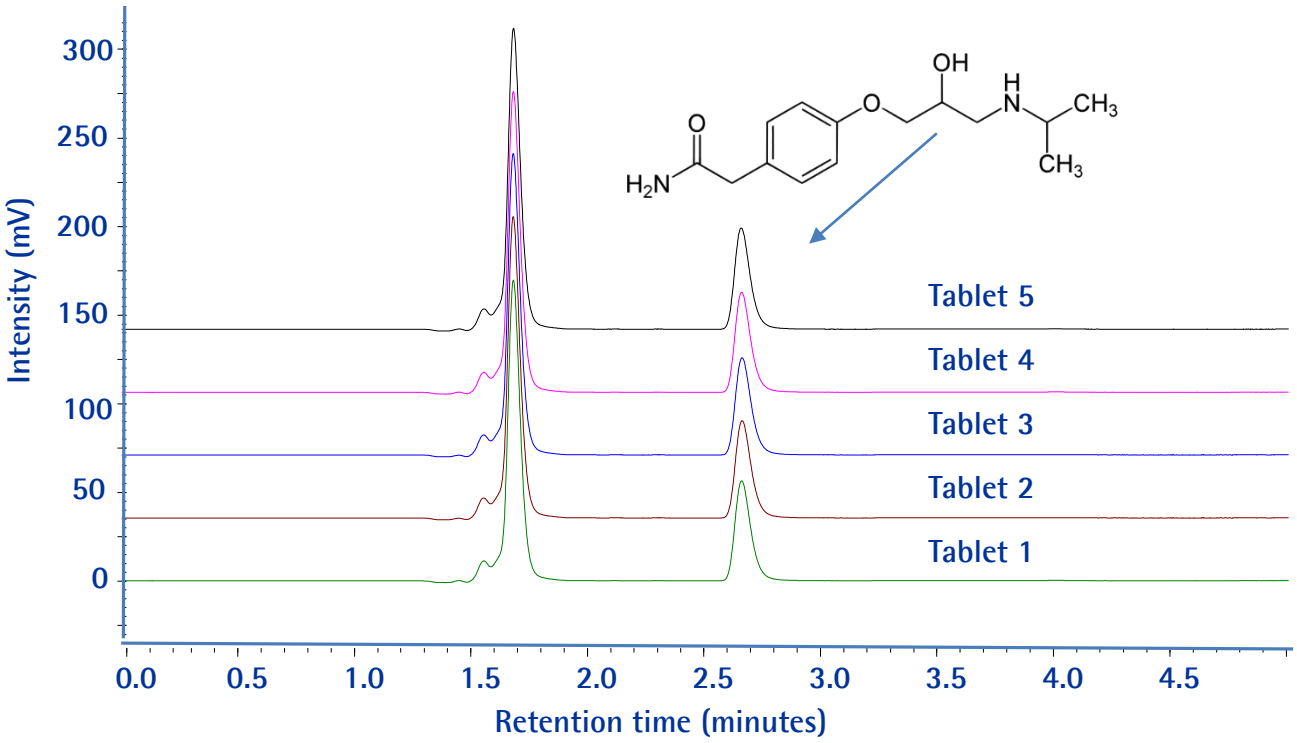


Chromatographic Data:

No.	Compound	Retention Time (min)	Tailing factor	Theoretical Plate
1	Atenolol	2.6	1.3	6500

Atenolol Tablets (USP)

– Chromolith® HighResolution RP-18 endcapped



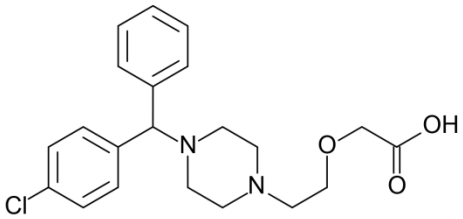
Result (%) = (rU/rS) ×CS× V × D × (100/L)

Peak Response		CS (Weight of Standard)	V Media Volume	L 100/Label claim	Dissolution
RU	RS	(mg/mL)	(mL)		(%)
257926	267164	0.012	900	8	83,4
258922	267134	0.012	900	8	83,7
259695	263980	0.012	900	8	85.0
258379	263852	0.012	900	8	84,6
258652	264069	0.012	900	8	84,6
Average					84.3±0.7

* Label claim of the Atenolol Tablet = 12.5 mg and D = 1 (Dilution Factor)

Cetirizine Hydrochloride (USP)

– Tablets



Cetirizine is a second-generation antihistamine.
Used in the treatment of hay fever, allergies, and angioedema
Cetirizine is a major metabolite of hydroxyzine, and a racemic selective H1 receptor antagonist.
Commercial brand names Zyrtec, Reactine...

Drug dissolution testing has been carried out following the experimental conditions in the USP37-NF32 monograph for Cetirizine Hydrochloride Tablets (using an isocratic HPLC method with RP-18 endcapped columns and thus scalable). A 250x4.6 mm column is prescribed with 5 µm L1 packing operating at 1.0 mL/min. To improve sample throughput we have transferred this method to a 100x4.6 mm long monolithic column.

The new method turned out to be faster, having improved chromatographic resolution, lower column backpressure, and still meeting all method performance criteria compared to the prescribed column.

Cetirizine Hydrochloride (USP)

– Tablets

Dissolution <711>

HPLC

Test 1
Medium: Water; 900 mL, degassed
Apparatus 2: 50 rpm
Time: 30 min

Buffer: 2.9 mL/L of phosphoric acid in water
Mobile phase: Acetonitrile and Buffer (2:3)
Standard solution: 11 µg/mL of USP Cetirizine Hydrochloride RS in water.
(This solution can be stored for 48 h at room temperature.)
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45 µm pore size.

Chromatographic system (see Chromatography 621, System Suitability.)
Detector: UV 230 nm
Column: 250x4.6 mm column; 5-µm packing L1
Flow rate: 1 mL/min
Injection volume: 50 µL
Run time: 1.3 times the retention time of cetirizine

System suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) 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 USP Cetirizine Hydrochloride RS in the Standard solution (mg/mL)
L = label claim (mg/Tablet)
V = volume of Medium, 900 mL

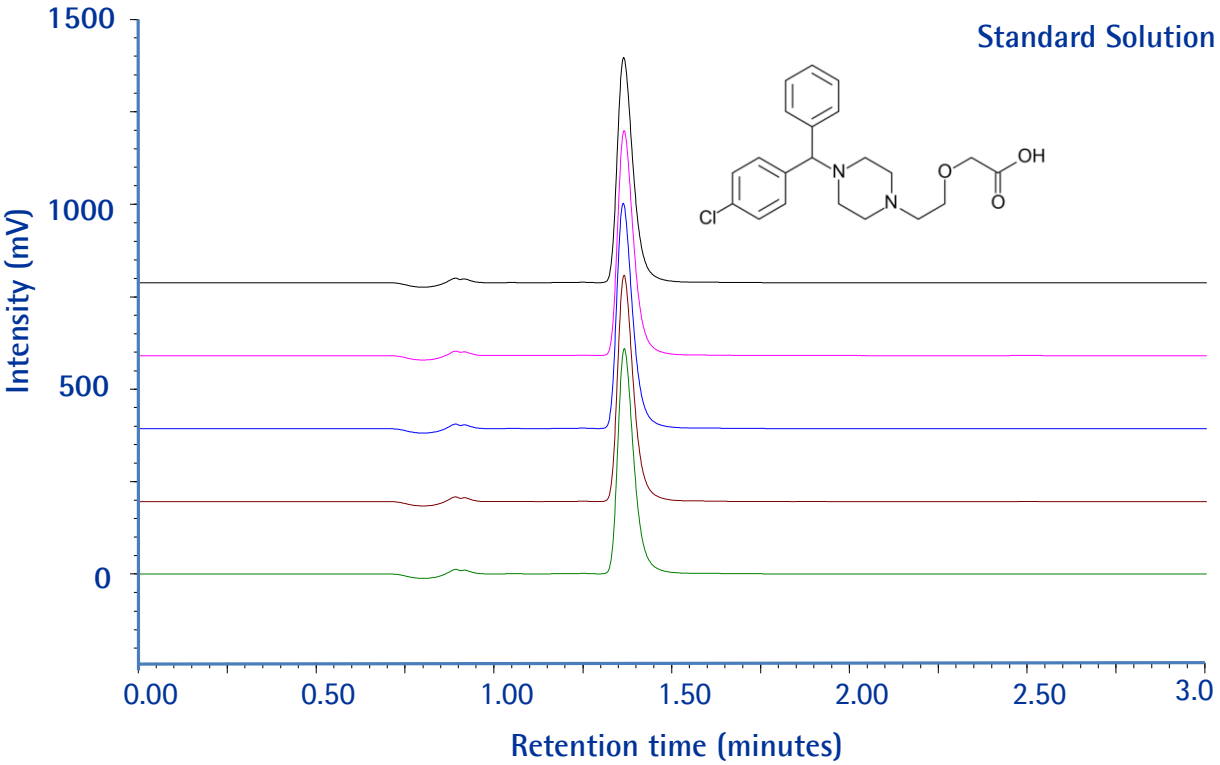
Tolerances: NLT 80% (Q) of the labeled amount of **cetirizine hydrochloride** ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.

Cetirizine Hydrochloride Tablets (USP)

– Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18e, 100x4.6 mm	1.52022.0001
Injection:	10 µl	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1 mL/min	
Mobile Phase:	Buffer: 2.9 mL/L of phosphoric acid in water. Mix acetonitrile and buffer 2:3 (v/v)	
Temperature:	35 °C	
Diluent:	Water 900 ml , 50 rpm for 30 minutes	
Sample soltion:	Filter the solution through 0.45 µm Millex HV Filter (PVDF Filter)	
Pressure Drop:	92 Bar(1334psi)	

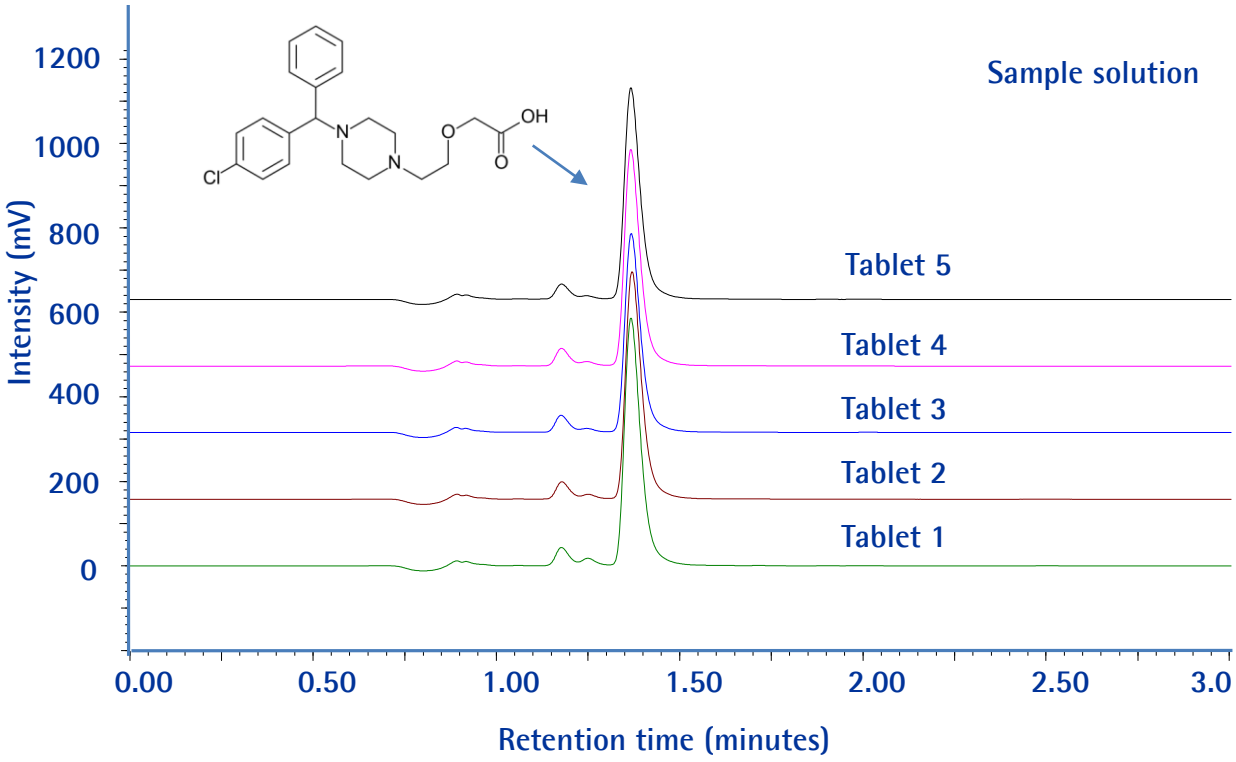


Chromatographic Data :

No.	Compound	Retention Time (min)	Tailing factor	Theoretical Plate
1	Cetrizine Hydrochloride	1.4	1.4	4610

Cetirizine Hydrochloride Tablets (USP)

– Chromolith® HighResolution RP-18 endcapped

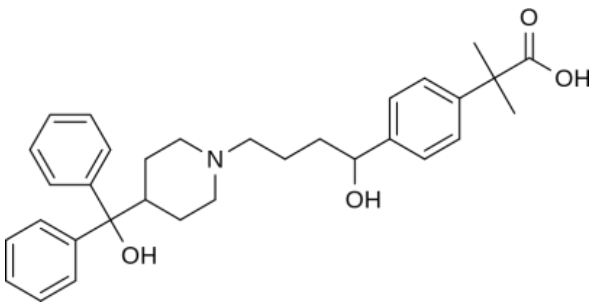


Result (%) = (rU/rS) ×CS× V × D × (100/L)

Peak Response		CS (Weight of Std)	V Media Volume	L* 100/Label claim	Dissolution
RU	RS	(mg/mL)	(mL)		(%)
1922106	1536929	0.012	900	8	108.0
1933635	1582924	0.012	900	8	105.5
1933035	1448987	0.012	900	8	115.3
1918776	1680700	0.012	900	8	98.6
1935359	1831921	0.012	900	8	91.3
Average					104±9

* Label claim of the Cetrizine HCl Tablet = 12.5 mg and D = 1 (Dilution Factor)

Fexofenadine Hydrochloride (USP)
– Tablets



Fexofenadine is an antihistamine pharmaceutical drug used in the treatment of allergy symptoms, such as hay fever, nasal congestion, and urticaria.

Common trade names are: Allegra, Fexidine, Telfast, Fastofen, Tilfur, Vifas, Telfexo, Allerfexo.

The current USP monograph method for dissolution testing of Fexofenadine tablets specifies the use of a 100x4.6 mm column with L1 (C18) packing as stationary phase. No particle size is mentioned wherefore the ratio must be calculated using the largest particle size consigned in the USP definition of the column, alternatively to comply with the system suitability criteria and provide adequate efficiency and resolution.

We have transferred this method to a monolithic column and the following pages illustrate that the acceptance criteras are being met for Fexofenadine dissolution Test 1 by following the experimental conditions in the USP37-NF32 monograph.

The new method is faster, having improved chromatographic resolution, and with lower column backpressure compared with a particle packed column.

Fexofenadine Hydrochloride (USP)
– Tablets

Dissolution <711> HPLC

Test 1
Medium: 0.001 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 50 rpm
Time: 10 and 30 min

Determine the percentages of the labeled amount of $C_{32}H_{39}NO_4 \cdot HCl$ dissolved by using the following method.

Solution A: 1.0 g of monobasic sodium phosphate, 0.5 g of sodium perchlorate, and 0.3 mL of concentrated phosphoric acid in 300 mL of water.
Mobile phase: Acetonitrile and Solution A (7:3)
Standard solution: USP Fexofenadine Hydrochloride RS in Medium to obtain a solution having a known concentration similar to that expected for the solution under test. *[NOTE—A small amount of methanol, not exceeding 0.5% of the total volume, can be used to dissolve fexofenadine hydrochloride.]*
System suitability solution: 0.44 mg/mL of USP Fexofenadine Related Compound A RS in water. Transfer 1.0 mL of this solution into a vial, and add 40 mL of the Standard solution.
[*NOTE—A small amount of acetic acid, not exceeding 5% of the total volume, can be used to dissolve fexofenadine related compound A.]*
Sample solution: Pass portions of the solution under test through 0.45-µm pore size glass fiber filter.

Chromatographic system (See Chromatography 621 , System Suitability.)
Detector: UV 220 nm
Column: 100x4.6 mm column; packing L1
Flow rate: 1 mL/min
Injection size: 2–3 µg column load of fexofenadine hydrochloride

System suitability
Samples: Standard solution and System suitability solution
Suitability requirements
Resolution: NLT 2.0 between fexofenadine and fexofenadine related compound A, System suitability solution. Relative standard deviation: NMT 2.0%, Standard solution

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of $C_{32}H_{39}NO_4 \cdot HCl$ dissolved in the portion of Tablets taken:

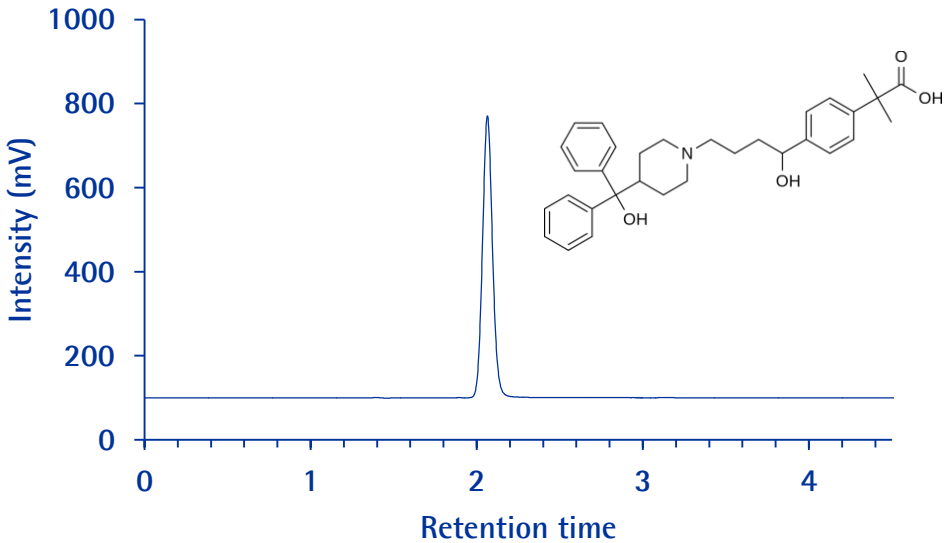
Result = (rU/rS) × (CS/L) × D × V × 100

Fexofenadine Tablet Dissolution (USP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Medium:	0.001 N hydrochloric acid; 900 mL	
Apparatus	USP Apparatus 2 (Paddle)	
Time:	10 minutes and 30 minutes	
Buffer:	1.0 g of monobasic sodium phosphate, 0.5 g of sodium perchlorate, and 0.3 mL of concentrated phosphoric acid in 300 mL of water	
Mobile Phase :	Buffer: Acetonitrile (30:70) (v/v)	
Temperature:	Ambient	
Sample prep:	50 ppm (0.05 mg/mL) Fexofenadine in medium	
Pressure Drop:	43 Bar (1957 psi)	

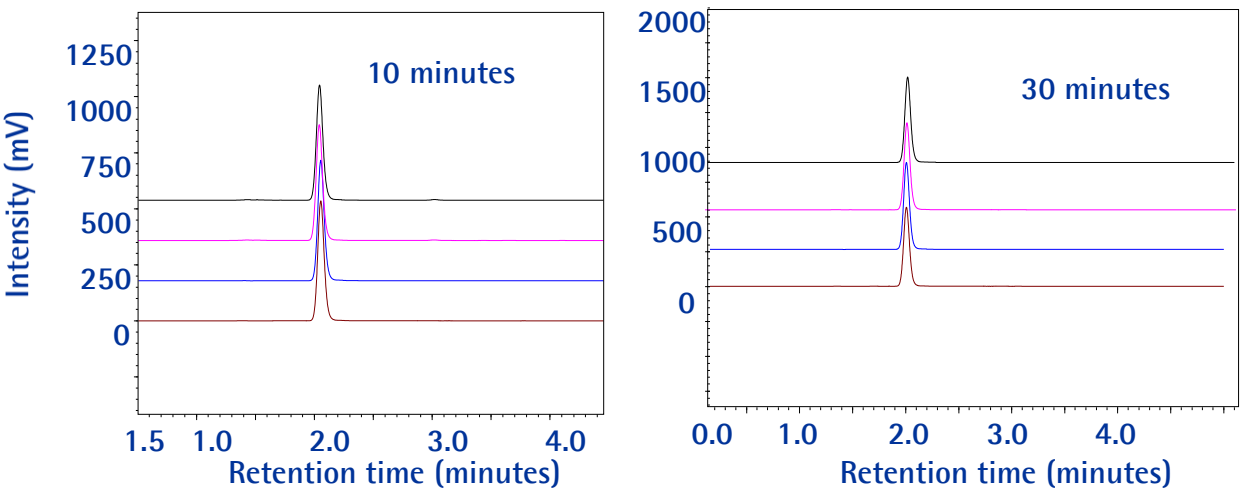


Chromatographic Data : Standard

No.	Compound	Retention Time (min)	Tailing factor	Theoretical plates
1	Fexofenadine	2.0	1.1	5592

Fexofenadine Tablet Dissolution (USP)

Chromolith® HighResolution RP-18 endcapped



Sample (area units)		Standard (area units)	[Standard] (mg/ml)	Label claim (mg/tablet)	Media (ml)	Dissolution (%)	
10 min	30 min					10 min	30 min
2108046	2439720	2804596	0.133	120	900	74.98	86.77
2101879	2444410					74.76	86.94
2069429	2438892					73.60	86.74
2073005	2436893					73.73	86.67
Average						**74.3**	**86.8**

Calculation of percentage Fexofenadine dissolved:

Result after 10 minutes = $(rU/rS) \times (CS/L) \times V \times 100$ = 74.3±0.6 % (n=4)

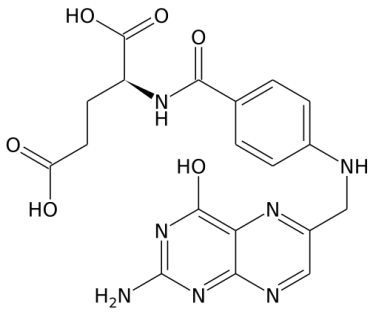
Result after 30 minutes = $(rU/rS) \times (CS/L) \times V \times 100$ = 86.8±0.1 % (n=4)

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*, 900 mL

Acceptance Criteria:

NLT 60% (Q) of the labeled amount of Fexofenadine is dissolved after 10 minutes;
NLT 80% (Q) of the labeled amount of Fexofenadine is dissolved after 30 minutes.

Folic Acid Tablets (USP)



Folic acid or folate is classified as a B vitamin (B9).
Folic acid is synthetically produced, and used in fortified foods and supplements.
Folate is converted by humans to dihydrofolate (dihydrofolic acid), tetrahydrofolate (tetrahydrofolic acid), and other derivatives, which have various biological activities.

Drug dissolution testing has been carried out following the experimental conditions in the USP37-NF32 monograph for Folic Acid Tablets (using an isocratic HPLC method with RP-18 endcapped columns and thus scalable). A 250x4.6 mm column is prescribed with L1 packing operating at 1.0 mL/min. To improve sample throughput we have transferred this method to a 100x4.6 mm long monolithic column.

The new method turned out to be faster, having improved chromatographic resolution, lower column backpressure, and still meeting all method performance criteria compared to the prescribed column.

Folic Acid Tablets (USP)

Dissolution <711>

HPLC

Test 1
Medium: Water; 500 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: Solution having a known concentration of USP Folic Acid RS, corrected for water content, in Medium
Sample solution: Filtered portion of the solution under test, suitably diluted with the Medium if necessary

Analysis
Samples: Standard solution and Sample solution
Proceed as directed in the Assay, making any necessary modifications.
Calculate the percentage of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) dissolved:

$$\text{Result} = (rU/rS) \times (CS \times D \times V/L) \times 100$$

rU = peak area of folic acid from the Sample solution
rS = peak area of folic acid from the Standard solution
CS = concentration of USP Folic Acid RS in the Standard solution (mg/mL)
D = dilution factor for the Sample solution
V = volume of Medium, 500 mL
L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) is dissolved

Assay
Mobile phase: Transfer 35.1 g of sodium perchlorate and 1.40 g of monobasic potassium phosphate to a 1-L volumetric flask. Add 7.0 mL of 1 N potassium hydroxide and 40 mL of methanol, dilute with water to volume, and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2.
Diluent: Aqueous solution containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL
System suitability solution: 0.2 mg/mL each of USP Folic Acid RS and USP Folic Acid Related Compound A RS in Diluent. [Note—Before use, pass through a filter of 1-µm or finer pore size.]
Standard solution: 0.20 mg/mL of USP Folic Acid RS, corrected for water content in Diluent
Sample solution: Equivalent to 0.2 mg/mL of folic acid, from NLT 20 powdered Tablets in Diluent; shake gently to aid dissolution, and filter, discarding the first portion.
Chromatographic system (see Chromatography 621, System Suitability.)
Detector: UV 254 nm
Column: 250x4.6 mm; packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL

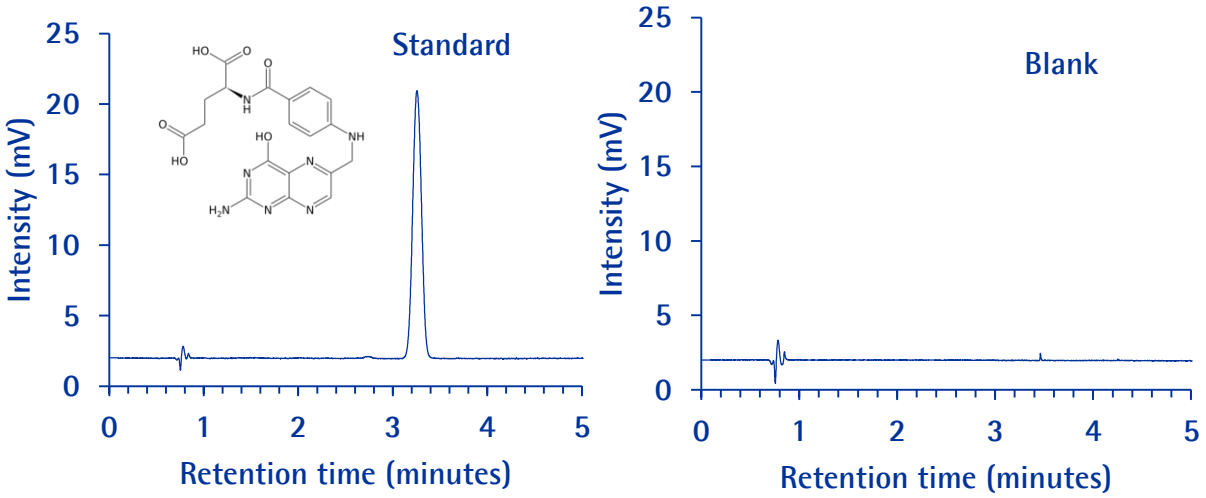
System suitability requirement
Resolution NLT 3.6 between folic acid related compound A and folic acid

Folic Acid Tablets (USP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP18e 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 254 nm	
Cell:	20 µL	
Flow Rate:	2.0 mL/min	
Medium:	Water; 500 mL	
Apparatus	USP Apparatus 2 (Paddle), 50 rpm	
Time:	45mins	
Mobile Phase:	Transfer 35.1 g of sodium perchlorate and 1.40 g of monobasic potassium phosphate to a 1.0 L volumetric flask. Add 7.0 mL of 1 N potassium hydroxide and 40 mL of methanol, dilute with water to volume and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2.	
Temperature:	Ambient	
Sample preparation:	10 ppm in diluent	
Pressure Drop:	130 Bar(1885 psi)	

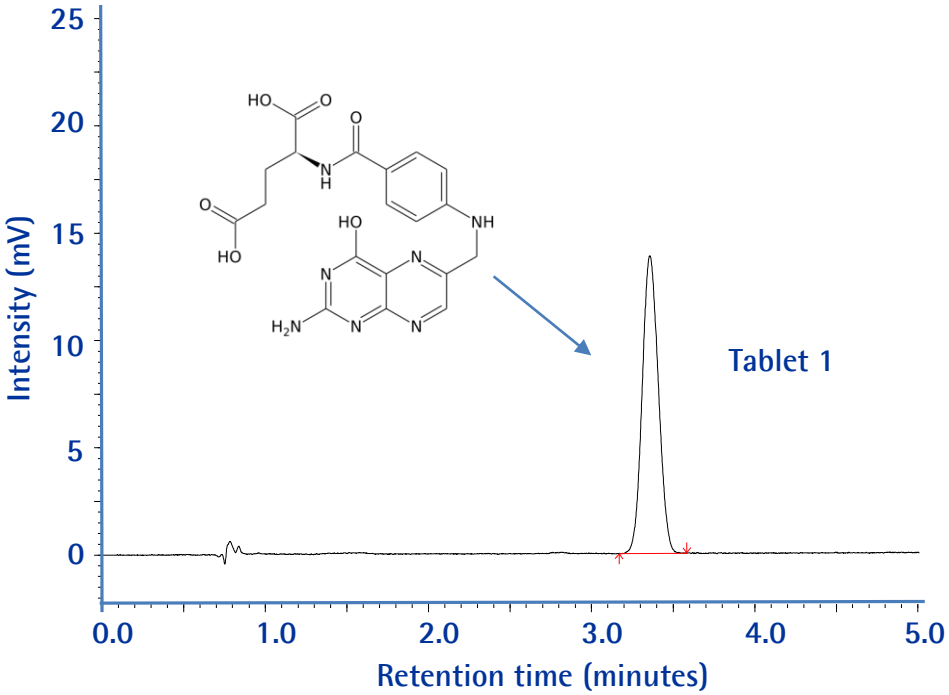


Chromatographic Data: Standard

No.	Compound	Retention Time (min)	Tailing factor
1	Folic acid	3.2	1.2

Folic Acid Tablets (USP)

Chromolith® HighResolution RP-18 endcapped

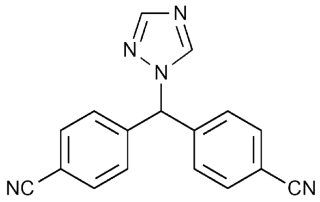


Result (%) = (rU/rS) × (CS × D × V/L) x 100

Area of Sample	Average area of Standard	CS (mg/ml)	Label claim (mg/tablet)	Dilution factor	V Media volume (ml)	Dissolution %
95753	122892	0.01	5	1	500	77.92
95451						77.67
95553						77.75
95620						77.81
95634						77.82
Average						77.8±0.1

Tolerance: NLT 75% (*Q*) of the labeled amount of folic acid is dissolved.

Letrozole Tablets (USP)



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.

Original Manufacturer:	Novartis (patent expired 2011)
Brand Name:	Femara

Drug dissolution testing has been carried out following the experimental conditions in the USP37-NF32 monograph for Letrozole Tablets (*using an isocratic HPLC method with RP-18 endcapped columns and thus scalable*).

We have transferred this method to a monolithic column. The new method is fast, having improved chromatographic efficiency, lower column backpressure, and still meeting all method performance criteria compared to the prescribed column.

Letrozole Tablets (USP)

Dissolution <711>

HPLC

Test 1
Medium: 0.1 N hydrochloric acid; 500 mL
Apparatus 2: 100 rpm
Time: 30 min
Standard solution: Transfer USP Letrozole RS to a suitable volumetric flask, dissolve in acetonitrile equivalent to 10% of the final volume, and dilute with Medium to volume to obtain a solution of 0.05 mg/mL of letrozole. Dilute this solution with Medium to obtain a solution of 0.005 mg/mL of letrozole.
Sample solution: Centrifuge a portion of the solution under test at 4000 rpm for 5 min.
Mobile phase and Chromatographic system: Proceed as directed in the Assay, except use an injection volume of 200 µL.

Assay
Mobile phase: Acetonitrile and water (48:52)
Diluent: Acetonitrile and water (30:70)
Standard stock solution: 0.2 mg/mL of USP Letrozole RS in Diluent.
[Note—Dissolve letrozole in acetonitrile, and then dilute with water.]
Standard solution: 10 µg/mL of USP Letrozole RS in Mobile phase, from the Standard stock solution
Sample stock solution: Equivalent to 50 mg of letrozole from Tablets, in a 250-mL volumetric flask. Add 20 mL of water, and shake for 5 min to dissolve the Tablets. Add 75 mL of acetonitrile, shake for 30 min, and dilute with water to volume. Centrifuge a portion of the solution.
Sample solution: 10 µg/mL of letrozole in Mobile phase, from the Sample stock solution

Chromatographic system (See Chromatography 621 , System Suitability.)
Detector: UV 230 nm
Column: 125x4.6 mm; 5 µm packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
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 the labeled amount of letrozole ($C_{17}H_{11}N_5$) dissolved:

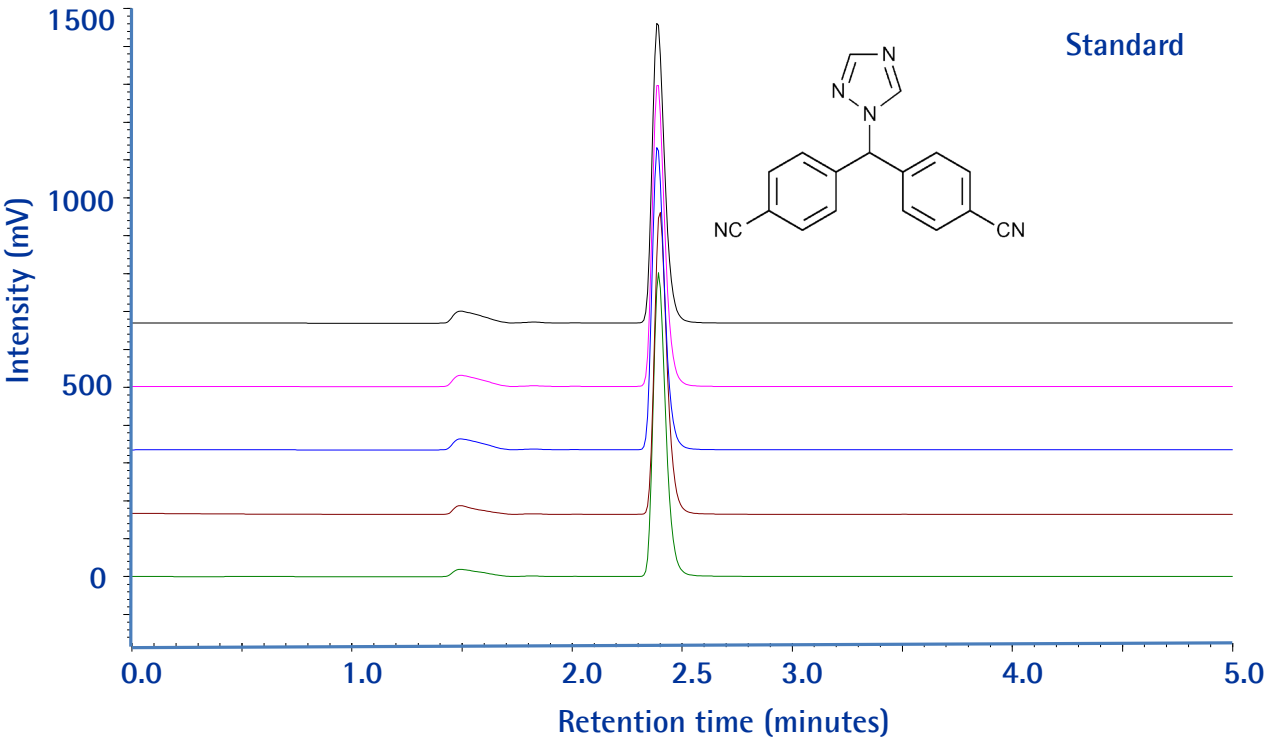
$$\text{Result} = (rU/rS) \times (CS/L) \times V \times 100$$

rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of USP Letrozole RS in the Standard solution (mg/mL)
L = label claim (mg/Tablet)
V = volume of Medium, 900 mL
Tolerances: NLT 80% (Q) of the labeled amount of letrozole ($C_{17}H_{11}N_5$) is dissolved.

Letrozole Tablets (USP)

Chromolith® HighResolution RP-18 endcapped

Column:	Chromolith® HighResolution RP-18e, 100x4.6 mm	1.52022.0001
Injection:	10 µl	
Detection:	Shimadzu 2010CHT, UV 230 nm	
Cell:	10 µL	
Flow Rate:	1 mL/min	
Mobile Phase:	Acetonitrile and water 48:52 (v/v)	
Temperature:	25 °C	
Diluent:	0.1 N HCl 500 ml , 100 rpm for 30 minutes	
Sample soltion:	Filter the solution through 0.45 µm Millex HV Filter (PVDF Filter)	
Pressure Drop:	92 Bar (1334psi)	



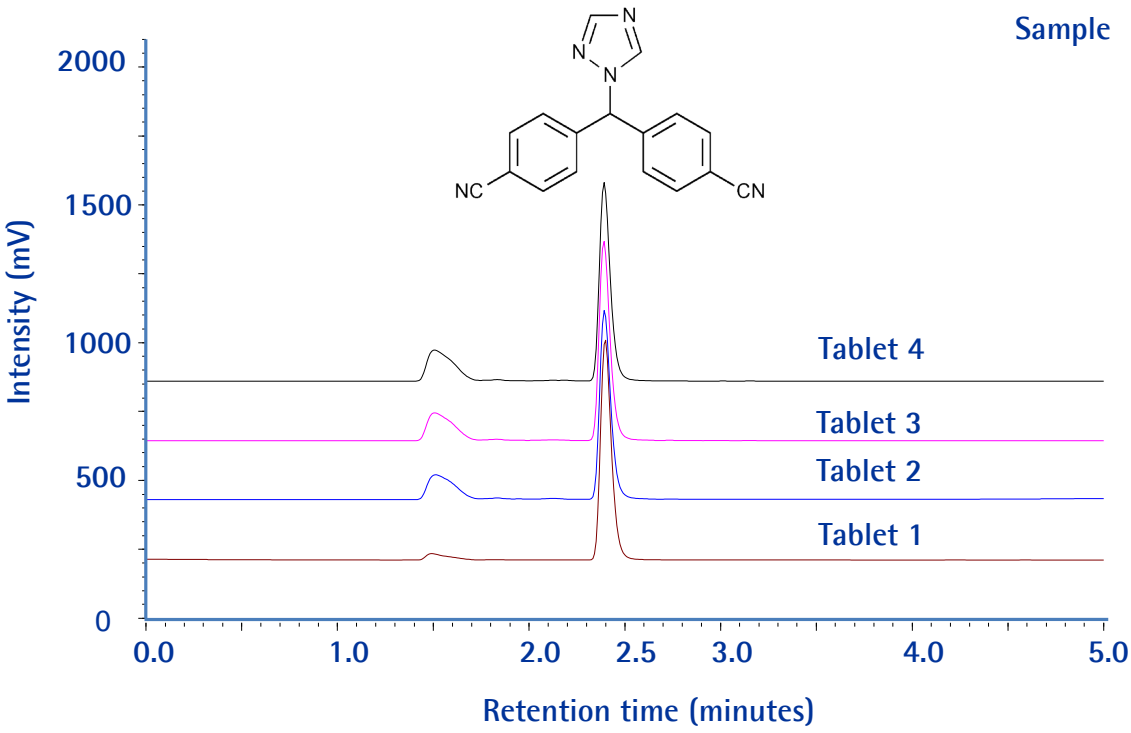
Suitability requirements
Tailing factor: 0.8-1.5

Chromatographic Data :

No.	Compound	Retention Time (min)	Tailing factor	Theoretical Plate
1	Letrozole	2.38	1.4	6500

Letrozole Tablets (USP)

Chromolith® HighResolution RP-18 endcapped



Result = (rU/rS) × (CS/L) ×V× 100

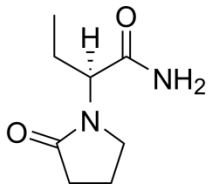
RU	RS	CS	V	L	Dissolution (%)
3151347	2901103	0.005	500	40	92
3183314	2884977	0.005	500	40	91.4
3164150	2911107	0.005	500	40	92.2
3202526	2784812	0.005	500	40	88.2
3205292	2911400	0.005	500	40	92.2
Average					91.2±1.7

Tolerances: NLT 80% (Q) of the labeled amount of Letrozole (C17H11N5) is dissolved.

* Label claim of the Letrozole Tablet = 2.5 mg

Levetiracetam (USP)

– Tablets



Levetiracetam is an anticonvulsant medication used to treat epilepsy.
It is the S-enantiomer of etiracetam.

Levetiracetam is marketed under the trade name Keppra by UCB Pharmaceuticals Inc., and from 2008 as a generic by many different companies under Levetiracetam.

Drug dissolution testing has been carried out following the experimental conditions in the USP37-NF32 monograph for Amoxicillin and Clavulanate Potassium Tablets (*using an isocratic HPLC method with RP-18 endcapped columns and thus scalable*).

We have transferred this method to a monolithic column. The new method is fast, having improved chromatographic efficiency, lower column backpressure, and still meeting all method performance criteria compared to the prescribed column.

Levetiracetam (USP)
– Tablets

Dissolution <711>

HPLC

Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: See Table 1.

Buffer: 6.8 g/L of monobasic potassium phosphate, adjusted with dilute potassium hydroxide to pH 5.6
Mobile phase: Acetonitrile and Buffer (15:85)
Standard solution: (L/1000) mg/mL in Medium, where L is the Tablet label claim, in mg
Sample solution: Pass a portion of the solution under test though a suitable filter of 0.45-µm pore size.

Chromatographic system (See Chromatography 621 , System Suitability.)
Detector: UV 220 nm
Column: 150x4.6 mm; 5-µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability (Sample: Standard solution)

Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) 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 USP Levetiracetam
RS in the Standard solution (mg/mL)
L = label claim (mg/Tablet)
V = volume of Medium, 900 mL

Tablet Strength (mg/Tablet)	Time (min)
250	15
500	15
750	15
1000	30

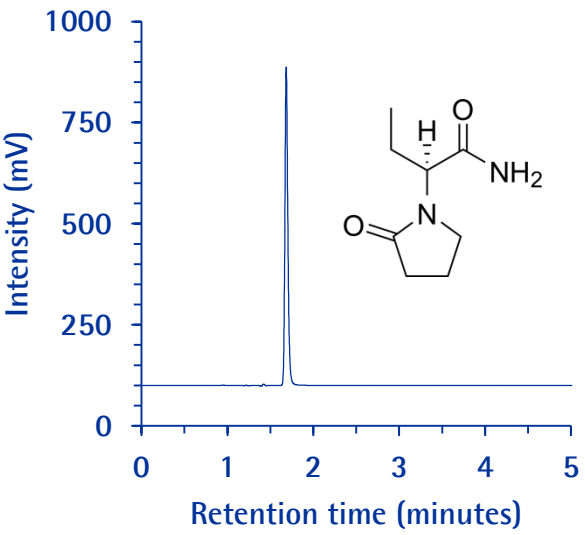
Tolerances
NLT 70% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 15 min for Tablets labeled to contain 250, 500, or 750 mg; NLT 80% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 30 min for Tablets labeled to contain 1000 mg.

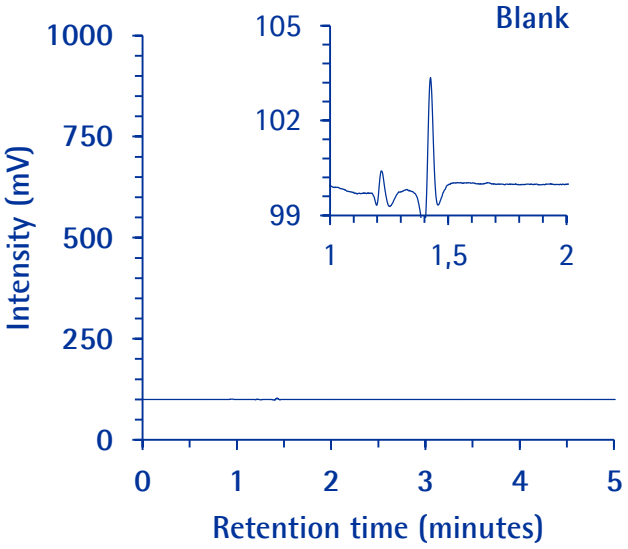
Levetiracetam Tablet Dissolution (USP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1.2 mL/min	
Medium:	Water	
Apparatus	USP Apparatus 2 (Paddle), 50 rpm	
Time:	15 minutes	
Buffer:	6.8 g/L of monobasic potassium phosphate, adjusted with dilute potassium hydroxide to a pH of 5.6	
Mobile Phase :	Buffer:Acetonitrile 85:15 (v/v)	
Temperature:	Ambient	
Sample:	500 ppm (0.5 mg/mL) of Levetiracetam in diluent	
Pressure Drop:	68 Bar (986 psi)	





Chromatographic Data: Standard

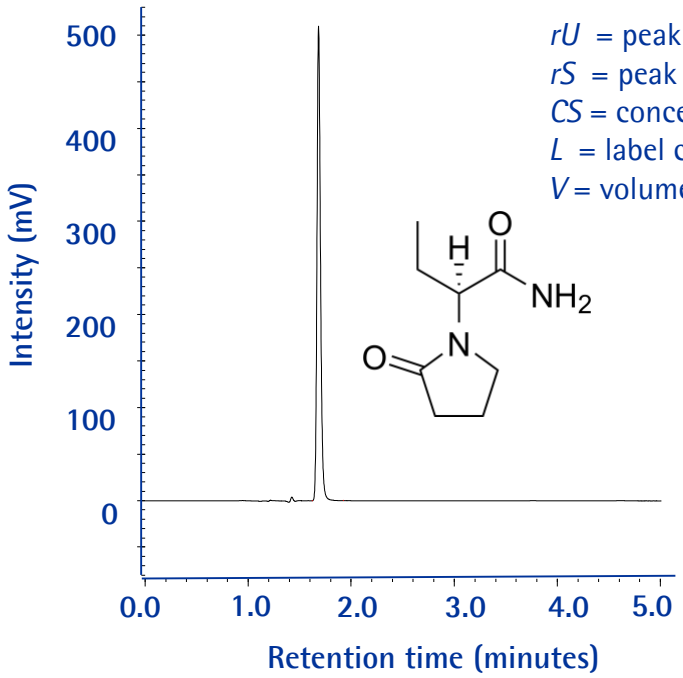
No.	Compound	Retention Time (min)	Tailing factor	Theoretical plates
1	Levetiracetam	1.6	1.2	11851

Levetiracetam Tablet Dissolution (USP)

Chromolith® HighResolution RP-18 endcapped

Calculation of percentage Levetiracetam dissolved:

Result = (*rU*/*rS*) × (*CS*/*L*) ×*V*× 100

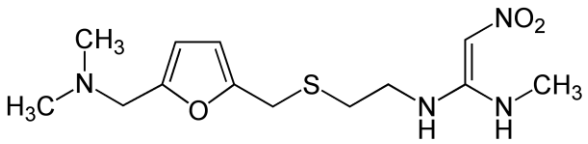


rU = peak response from the *Sample solution*
rS = peak response from the *Standard solution*
CS = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Sample (area units)	Standard (area units)	[Standard solution] (mg/ml)	Label claim (mg/tablet)	Media volume (ml)	Dissolution (%)
1718244	1870805	0.5	500	900	82.6
1771524					85.2
1727706					83.1
1732646					82.9
1710910					82.3
Average					83.2 ± 1.1

NLT 70% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 15 min for Tablets labeled to contain 250, 500, or 750 mg; NLT 80% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 30 min for Tablets labeled to contain 1000 mg.

Ranitidine Hydrochloride Tablets (USP)



Ranitidine is a histamine H2-receptor antagonist that inhibits stomach acid production.
It is used in treatment of peptic ulcer disease and gastroesophageal reflux disease.
Ranitidine is used alongside fexofenadine, and other antihistamines, for the treatment of hives and other skin conditions.

Common brand name: Zantac

Drug dissolution of ranitidine tablets is normally conducted by determining the amount of C13H22N4O3S dissolved with UV measurements at 314 nm using filtered portions of the solution under test in comparison with a standard solution with a known concentration of USP Ranitidine Hydrochloride RS in the same medium.

Here we present an alternative HPLC procedure based on the normal dissolution test and the assay test under Ranitidine Injection. In this new alternative method, drug binding has also been tested with Nylon, PTFE and PVDF syringe filters.

The new method proposal is very fast, with an analysis time less than 3 minutes.
It meets all system suitability requirements.
Drug dissolution has a tolerance higher than 80% after 45 minutes and with no prominent drug binding from the sample solution to any of the filters tested.

Ranitidine Hydrochloride Tablets (USP)

Dissolution <711>

Medium: water; 900 mL.
Apparatus 2: 50 rpm.
Time: 45 minutes.

Procedure: Determine the amount of $C_{13}H_{22}N_4O_3S$ dissolved from UV absorbance at the wavelength of maximum absorbance at about 314 nm using filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Ranitidine Hydrochloride RS in the same medium.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{13}H_{22}N_4O_3S$ is dissolved in 45 minutes.

HPLC

Assay (from Ranitidine Injection)
Mobile phase: Prepare a filtered and degassed mixture of methanol and 0.1 M aqueous ammonium acetate (85:15). Make adjustments if necessary (see System Suitability under Chromatography 621).
Standard preparation: Dissolve an accurately weighed quantity of USP Ranitidine Hydrochloride RS in Mobile phase to obtain a solution having a known concentration of about 0.112 mg (equivalent to 0.100 mg of ranitidine base) per mL.
System suitability solution: Dissolve accurately weighed quantities of USP Ranitidine Hydrochloride RS and USP Ranitidine Related Compound C RS in Mobile phase to obtain a solution having known concentrations of about 0.112 mg per mL and 0.01 mg per mL, respectively.
Assay preparation: Dilute an accurately measured volume of Injection, quantitatively and stepwise if necessary, with Mobile phase to obtain a solution having a concentration of 0.1 mg of ranitidine per mL.

Chromatographic system (see Chromatography 621)

Detection: UV at 322 nm
Column: a 4.6 mm × 20 to 30 cm column, packing L1.
Flow rate: about 2 mL per minute.

Suitability requirements
Tailing factor: NMT 2.0
Column efficiency: NLT 700 theoretical plates (measured from the ranitidine hydrochloride peak)
Relative standard deviation: NMT 2.0%

Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the resolution, R, between ranitidine hydrochloride and N-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]sulfinyl]ethyl]-N-methyl-2-nitro-1,1-ethenediamine (ranitidine related compound C) is not less than 1.5.
Chromatograph the Standard preparation, and record the peak responses as directed for Procedure.

Ranitidine Hydrochloride Tablets (USP)

Dissolution <711> HPLC

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 area responses for the major peaks.

Calculate the quantity, in mg, of C13H22N4O3S in the portion of Injection taken by the formula:

(314.40 / 350.87)(L / D)(C)(rU / rS)

where 314.40 and 350.87 are the molecular weights of ranitidine and ranitidine hydrochloride, respectively

L is the labeled quantity of ranitidine in the Injection taken;
D is the concentration, in mg per mL, of ranitidine in the Assay preparation on the basis of the labeled quantity and the extent of dilution;

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

The new method proposal include the following:

Chromatographic system
Detection: UV at 314 and 322 nm
Column: a 100x4.6 mm column, packing L1.
Flow rate: about 1.0 mL/minute.
Filter: Either Millex PVDF, Millex PTFE or Millex Nylon

The drug recovery after dissolution was calculated according to the following equation:

$$Result = (rU/rS) \times (CS/L) \times V \times 100$$

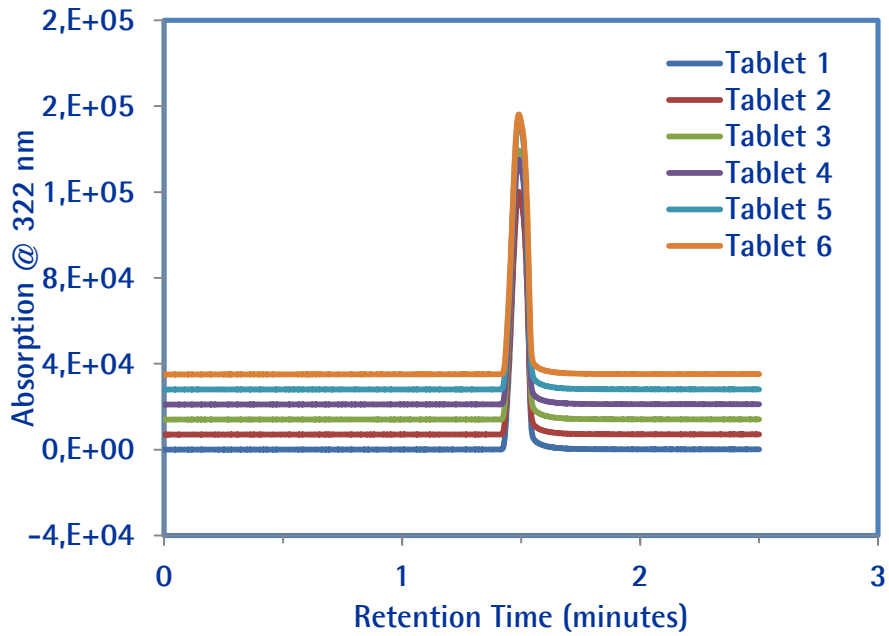
rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of the Standard solution (mg/mL)
L = label claim (mg/Capsule)
V = volume of Medium, 900 mL

Ranitidine Hydrochloride Tablets (USP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	2 µl	
Detection:	UV detection @ 314 nm and 322 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Medium:	Water, 900 ml for 45 min	
Apparatus 2:	50 rpm	
Mobile phase:	0.1 M Ammonium acetate in water + methanol 15:85 (v/v)	
Temperature:	35°C	
Diluent:	Mobile phase	
Standard Solution:	Dissolve accurately weighed quantity of Ranitidine Hydrochloride USP in mobile phase. Dilute as necessary.	
Sample Solution:	Filter portions of solution under study using appropriate 0.45 µm pore size syringe filter	
Pressure Drop:	173 bar (2500 PSI)	

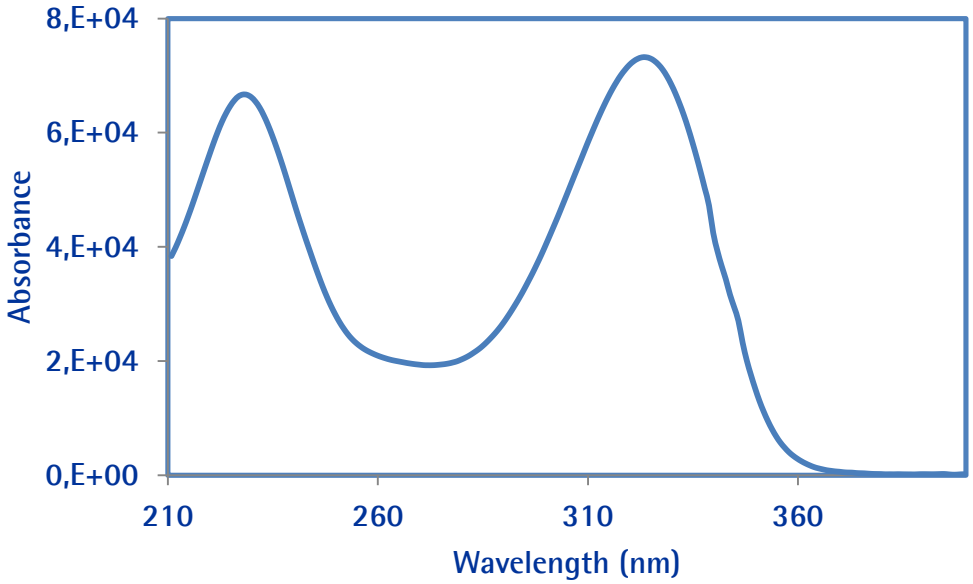


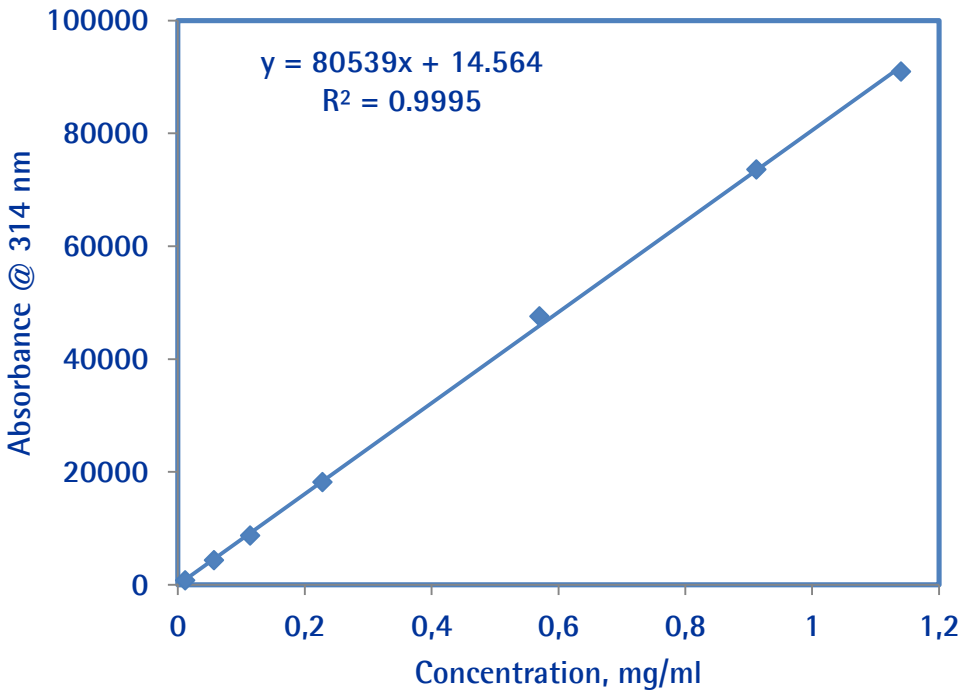
Chromatographic Data: Standard

No.	Compound	Retention Time (min)	Tailing factor	Theoretical plates
1	Ranitidine hydrochloride	1.48	1.00	1557

Ranitidine Hydrochloride Tablets (USP)

Chromolith® HighResolution RP-18 endcapped





Ranitidine Hydrochloride Tablets (USP)

Chromolith® High Resolution RP-18 endcapped

Result = (rU/rS) × (CS/L) ×V× 100

Filtrate Used	Syringe Filter Used		
	Millex PTFE	Millex Nylon	Millex PVDF
1st ml	107.6	122.8	106.8
2nd ml	117.5	120.8	122.5
3rd ml	119.6	121.6	122.7
5th ml	118.1	121.0	122.6

Did you know?

A quick way to speed-up the preparations for your dissolution tests and to improve the precision in your results is to utilize the automatic volumetric water dispense function on modern Milli-Q water systems.

Automatic volumetric water dispensing
Volumetric water dispensing is set on the base of the POD unit.
You can adjust the volume to be delivered with the (+) and (-) keys, and then press the volumetric dispensing button to start delivery of the selected volume, with excellent accuracy (< 1 %) and reproducibility (cv < 1 %).

The mast and the arm supporting the Q-POD® and E-POD® dispensers are designed to accommodate all commonly used glassware — from a 250 mL Erlenmeyer flask to a 5 L calibrated flask — and even a 20 L carboy!

For hands-free water delivery, an optional footswitch can be connected to the base of the POD dispensers or directly to the Milli-Q® Integral system. Press once to start and once to stop.

Solvents and Reagents

Product	P/N
Chromatography	
Chromolith® High Resolution RP-18 endcapped, 100x4.6 mm	1.52022
Solvents	
Acetonitrile (isocratic grade for liquid chromatography) LiChrosolv®	1.14291
Methanol (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur	1.06007
Methanol for liquid chromatography LiChrosolv®	1.06018
Water for chromatography*	1.15333
* or use a Milli-Q Integral Water Purification System	
Ion-pair reagents	
1-Heptanesulfonic Acid, Sodium Salt, Monohydrate	HX0101
Dibutylamine	8.03222
Filters	
Millex-LCR Filter, 0.45 um, Hydrophilic PTFE membrane, 25 mm, non-sterile:	SLCR25NB
Millex-HV 0.45 µm, PVDF, 33 mm, non-sterile	SLHV033NB
Millex-HN, 0.45 µm, nylon, 33 mm, non-sterile	SLHN033NS

Solvents and Reagents

Product	P/N
Buffer Salts	
Ammonium acetate for analysis EMSURE® ACS,Reag. Ph Eur	1.01116
Sodium acetate anhydrous for analysis EMSURE® ACS,Reag. Ph Eur	1.06268
Sodium perchlorate monohydrate for analysis EMSURE®	1.06564
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur	1.06342
di-Sodium hydrogen phosphate dihydrate for analysis EMSURE®	1.06580
tri-Sodium phosphate dodecahydrate for analysis EMSURE® ACS,Reag. Ph Eur	1.06578
Potassium dihydrogen phosphate for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.04877
Acids	
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00063
Hydrochloric acid (fuming 37% for analysis EMSURE® ACS,ISO,Reag. Ph Eur)	1.00317
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00573
Bases	
GR ACS Ammonium Hydroxide	AX1303
Potassium hydroxide solution 47% for analysis EMSURE®	1.05545
Sodium hydroxide solution 50% for analysis EMSURE®	1.58793