How to use Monolithic columns in Regulated pharmaceutical Analysis

Scaling of monograph methods

2015-4

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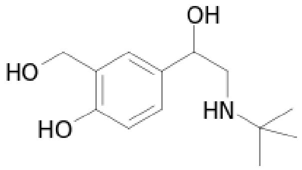
Content

Molecular Structures



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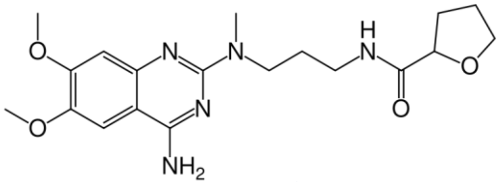

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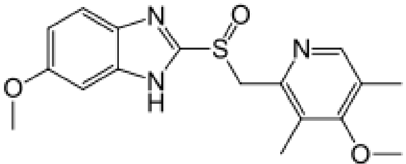

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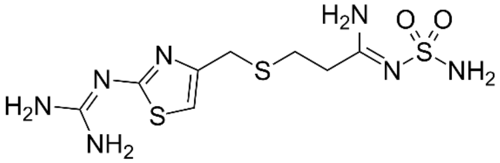

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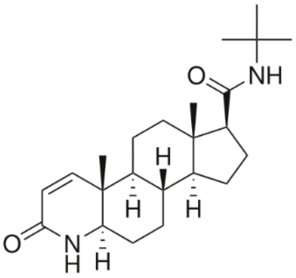

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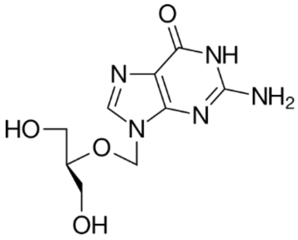
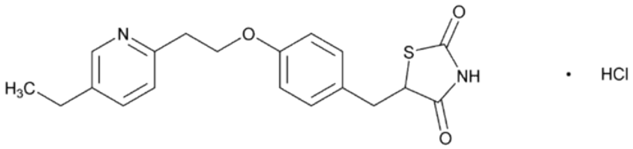


Ganciclovir



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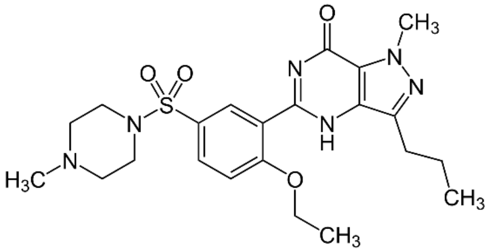
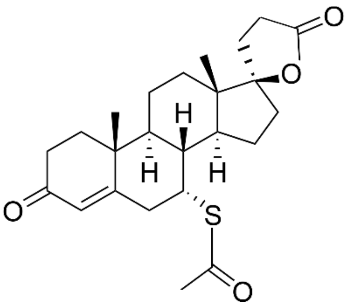
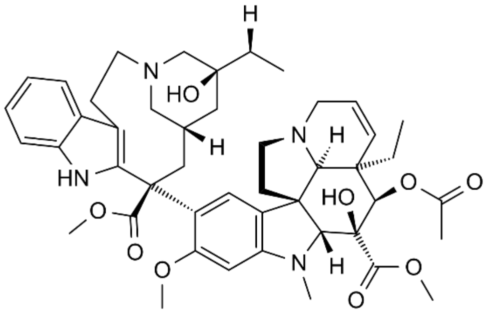


Sildenafil



Spironolactone



Vinblastine

Application Index

Introduction

This compilation focus on United States pharmacopeia (USP) monograph methods, and how method transfer of isocratic HPLC methods is carried out from particulate to monolithic columns following the updated USP general chapter 621.

In all examples we have used modern particle packed HPLC columns and monolithic Chromolith® HighResolution RP-18 endcapped columns, Lichrosolv® high purity solvents (isocratic grade) and Emsure® high purity reagents.

Select the Analytical Tools with the Monograph in Focus

instrument ?
detection ?

sample preparation?

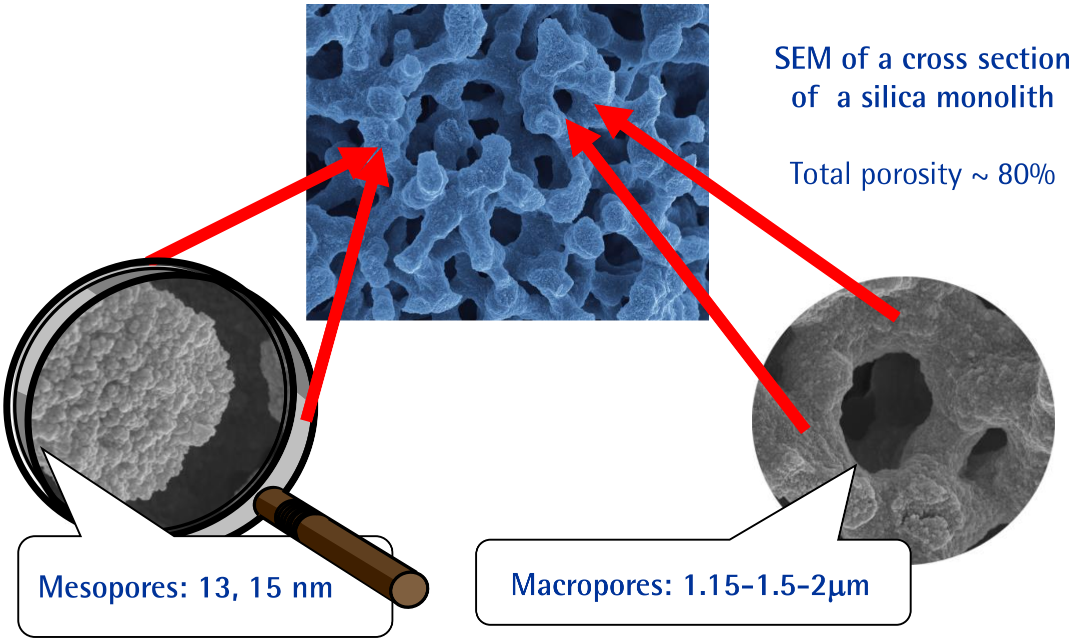
Monograph

reagents ?
solvents ?

column?

Monolithic Silica Columns
- With Rigid Bimodal Pore Structure

Chemically the same as high purity silica particles



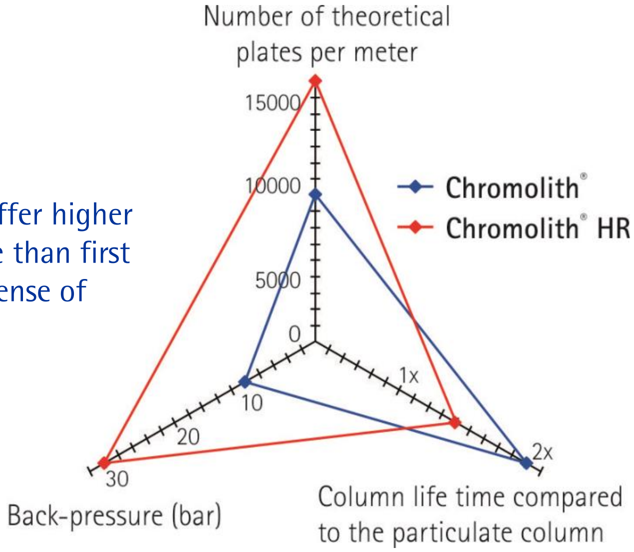
SEM of a cross section of a silica monolith

Total porosity ~ 80%

Mesopores: 13, 15 nm

Macropores: 1.15-1.5-2µm

Chromolith® vs Chromolith® HighResolution



Chromolith® HighResolution

The new Chromolith® HighResolution family offer higher separation efficiency and improved peak shape than first generation monolithic columns but at the expense of slightly higher column back-pressure.

USP Packings (L classifications)

Merck Millipore Monolithic 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)
L8	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

As of November 2014 the USP has extended the general description to include "or a monolithic rod" to the L8, L10, L11 and L20 packings definition. This update is offical and published in the pharmacopeial forum (PF) 40(6). It will be implemented in USP39-NF34 that comes active in 2016, but you can already now utilise it when transferring your methods if you refer to this coming change.

Register free of charge at the pharmacopeial forum (PF):
http://www.usppf.com

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 downtime during washing and re-equilibration of the column due to sample matrix issues.

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 even at high linear velocities, still at low column back pressure. You can thus vary the flow rate over a large range even when you analyze “dirty” samples; i.e samples from dissolution testing which are only filtered and considerably more “matrix rich” than standard solution samples and/or samples that has gone through sample clean-up before the HPLC analysis. If you have sufficient chromatographic space ,i.e. resolution between your peaks, you can easily increase the flow rate and save time.

The higher mass loadability compared with solid-core particle columns is another benefit with monolithic columns, exemplified on page 10, a valuable feature in impurity profiling methods.

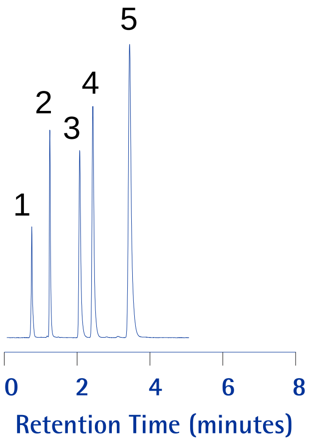
Chromolith® HighResolution columns provide:

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

High Efficiency and Symmetrical Peaks

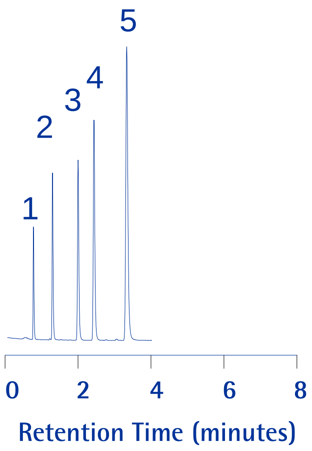
Chromolith® Performance
RP-18 endcapped 100x4.6mm

N/m	101,300
TUSP	1.49
Pressure	21 bar



Chromolith® HighResolution
RP-18 endcapped 100x4.6mm

N/m	164,900
TUSP	1.19
Pressure	57 bar



Mobile phase: Acetonitrile and water (60:40)
Flow rate: 2 mL/min
Pressure drop: 71 bar (1022 psi)
Temperature: Ambient
Detection: UV 254 nm
Injection: 5 µL

Sample:
1. Urea
2. Biphenyl-2-ol
3. Progesterone
4. Hexanophenon
5. Anthracene

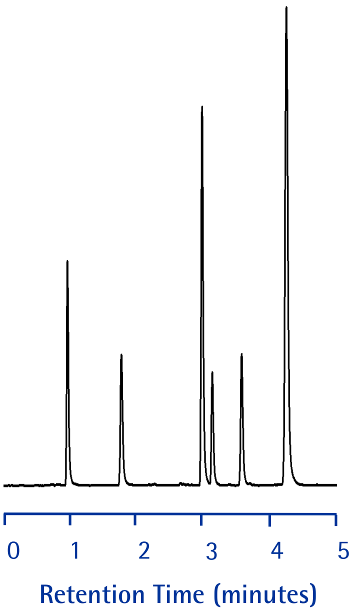
Symmetrical Peaks for Basic Compounds

Chromolith® HighResolution

Mobile phase A: Acetonitrile
B: 20 mM NaH_2PO_4, pH 7.6
Flow rate: 2 mL/min
Gradient: See table
Pressure drop: 63-69 bar (907-994 psi)
Temperature: Ambient
Detection: UV 254 nm
Injection: 1 µL

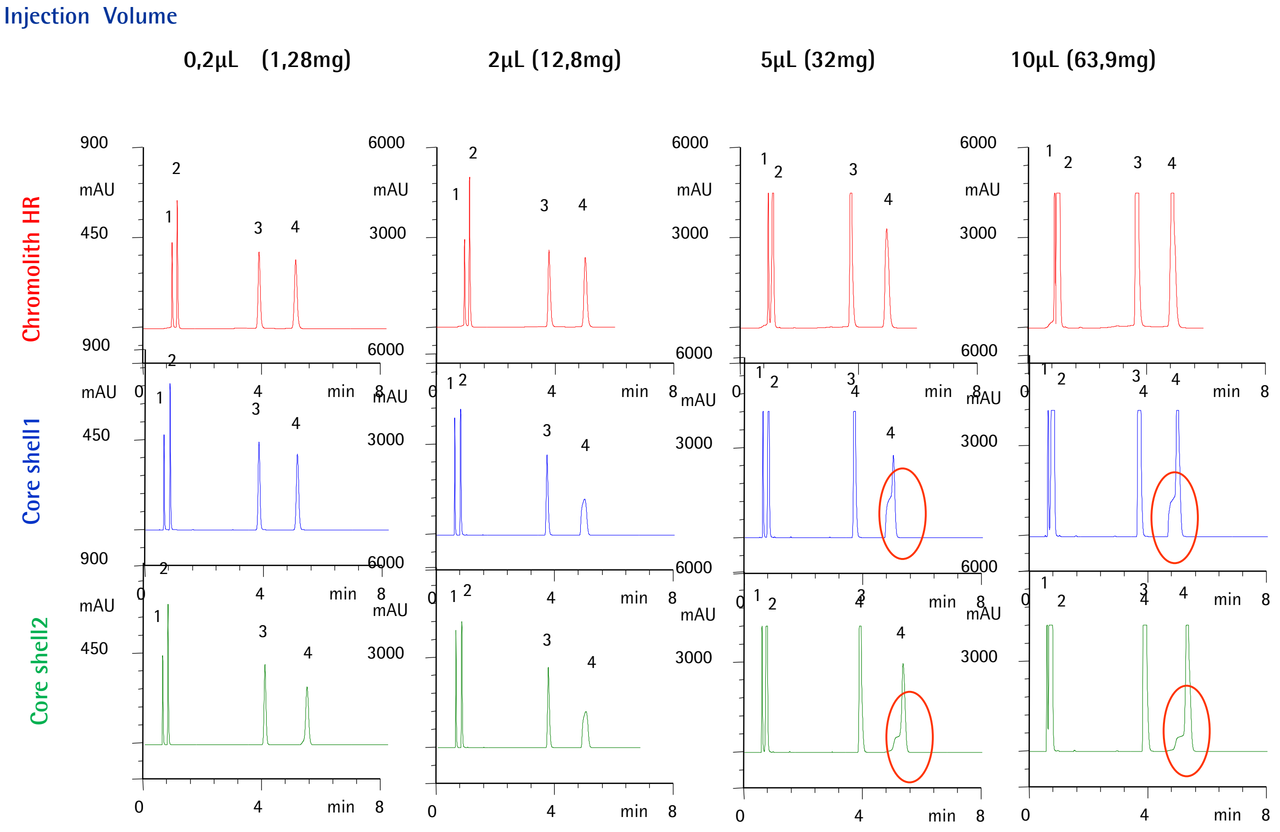
Sample:
1. Caffeine (125 µg/ml)
2. Aniline (113 µg/ml)
3. N-Methylaniline (77 µg/ml)
4. 2-Ethylaniline (83 µg/ml)
5. 4-Nitranisole (208 µg/ml)
6. N,N-Dimethylaniline (100 µg/ml)

Time (min)	A (%)	B (%)
0	100	0
0.5	45	55
7	45	55



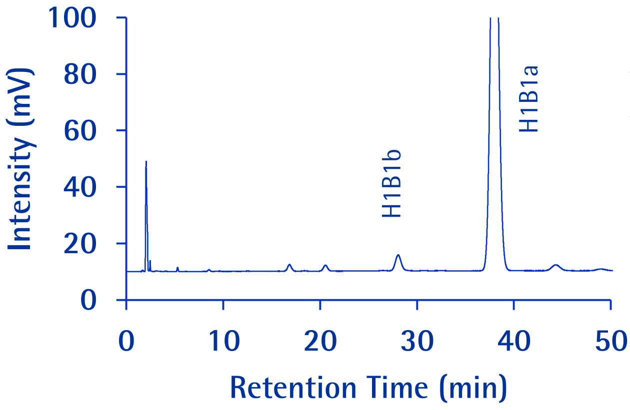
Higher Mass Loadability Columns

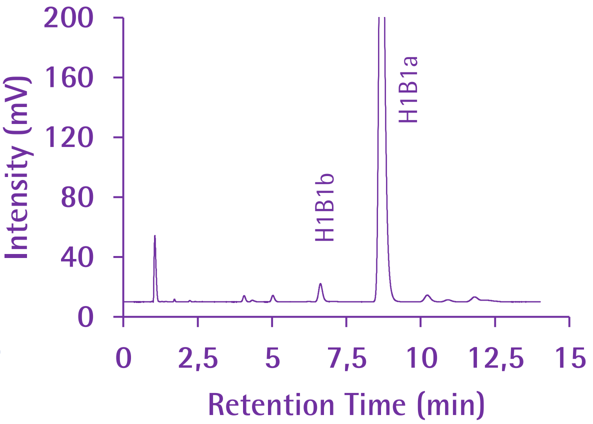
Loadability (sample capacity)



Mobile phase (v/v):	Acetonitrile/Water 25:75	Flow:	1.5 mL/min
Injection volume:	See figure	Detection:	UV 220 nm
Temperature:	Ambient		
Sample:	1. 27.7 mg Uracil 2. 28.1 mg Caffeine 3. 110.0 mg Acetophenone 4. 63.9 mg Hexobarbiturate Dissolved in 10 mL acetonitrile/water 40:60 v/v		

You can use the same HPLC system!





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)

Example: Ivermectin USP method

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 with the Ivermectin USP method transfer.

Impurity Profiling Methods

Various regulatory authorities like International conference of harmonization (ICH), European Directorate for the Quality of Medicines and Healthcare (EDQM), United States Food and Drug Administration (USFDA), and Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's).

Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research and manufacturing. Identification of impurities is carried out with various analytical techniques, and where different chromatographic and spectroscopic techniques are common. Either alone or in combination with other techniques.

There are different methods for detecting and characterizing impurities with thin layer chromatography (TLC), high performance thin layer chromatography HPTLC, gas chromatography (GC), high performance liquid chromatography (HPLC), and atomic absorption spectroscopy (AAS) besides more classical tests based on titration.

Especially HPLC has been widely exploited for impurity profiling methods, and reasons for this is the wide range of detectors available that connect easily with HPLC along with the variety of column chemistries (stationary phases) commercially available. In simple words, with HPLC it is possible to develop robust and reliable methods having necessary sensitivity and linearity, that meet requirement in selectivity and provide cost effectiveness to the laboratory.

On the following pages we have included several monograph methods with separations of different type of molecules; using reversed phase columns with RP-8 endcapped and RP-18 endcapped stationary phases and where the methods are running on columns with state-of-the-art monolithic backbones (Chromolith® HighResolution).

When working with monolithic columns you can expect a very high mass loadability.
You should be careful of not overloading with volume, especially if sample solvent is stronger than mobile phase, due to the higher porosity (~80%) created by the meso/macropore structure.

Recommended injection volume is 5-20 microliter, and with massload up to 100-200 mg/mL.

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 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 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.

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 described 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. Changes in the chemical characteristics (“L” designation) of the stationary phase are considered to be a major modification, unless the monograph has more than one packing mentioned, herein exemplified with the esomeprazole monograph.

Adjustments to the composition of the mobile phase in gradient elution may cause changes in selectivity and are not allowed.

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

	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.

"The situation with monolithic columns is similar to core shell columns. In those cases the chapter allows to make the adjustment using the calculation of N is within −25% to +50%, relative to the prescribed column in the particular procedure.

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.

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, Merck 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".*

At Merck Millipore we 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.

How to change and transfer a method?

The transfer of analytical procedures (TAP), also referred to as method transfer, is the documented process that qualifies a laboratory (the receiving unit) to use an analytical test procedure that originated in another laboratory (the transferring unit), thus ensuring that the receiving unit has the procedural knowledge and ability to perform the transferred analytical procedure as intended. TAP can be performed and demonstrated by several approaches, such as comparative testing, co-validation between two or more laboratories or revalidation/partial revalidation. For more details, please read further in USP general chapter <1224> Transfer of Analytical Procedures. Revalidation or partial revalidation is an acceptable approach for transfer of validated procedures if those characteristics described in USP chapter <1225> anticipated to be affected by the transfer are addressed.

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
- Recommends data for submission to USP-NF
- Guidance on which analytical performance characteristics are needed based on the type of test
- Incorporates ICH guidelines Q2A and Q2B

Compendial test requirements vary from highly exacting analytical determinations to subjective evaluation of attributes. Considering this broad variety, it is only logical that different test procedures require different validation schemes. This chapter covers only the most common categories of tests for which validation data should be required. These categories are as follows:

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

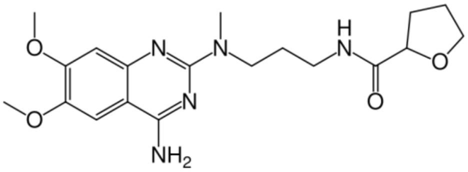
Why USP <1226> is needed:

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.

On the following pages we will use USP monograph methods as examples to illustrate what is allowed and how you can transfer your methods to more become more efficient and increase the productivity without changing your HPLC system.

Method Transfer – I

The following pages highlight an example where the USP monograph is first developed with a particle packed column fitting the monograph descripition and thereafter transferred to the same geometry (150x4.6 mm) monolithic column.



The current Alfuzosin Hydrochloride monograph serves as the example and where we focus at the organic impurities determination (related substances or impurity profile), method details are given on page 23.

The monograph specify a 150x4.6 mm column with L1 (RP-18) packing having 5 µm particles operating at 1.5 mL/min. When the particle size and/or column backbone is changed, the flow rate may require adjustment, for the same performance (as measured by reduced plate height). Flow rate changes can be made by using the equation on page 15 or it can be adjusted by ±50%.

The example with monolithic column, page 25-26 is within allowed changes using the calculation of N (within –25% to +50%, relative to the prescribed column) in the particular procedure when working at a flow rate of 1.0 mL/min. All performance criteria is fulfilled with both columns; RRT as well as peak-to-valley ratio.

The following benefits can be attained in transferring from particle to monolithic column:

a) The method runs faster. The retention is > 30% longer with the particle column.

b) Lower solvent consumption with the monolithic column
(the elution volume is 50% less for last eluting peak)

c) Lower backpressure on the monolithic column = less stress on HPLC system

Alfuzosin and Related Substances (USP38-NF33)

Monograph method for Alfuzosin Hydrochloride - Organic Impurities

(USP38–NF33 Page 2086 Pharmacopeial Forum: Volume No. 38(6)

Mobile phase: Acetonitrile, tetrahydrofuran, and Buffer (20:1:80)

Buffer: Dilute 5 mL of perchloric acid in 900 mL of water.
Adjust with 2 M sodium hydroxide to a pH of 3.5, and dilute with water to 1 L.

System suitability solution: 0.4 mg/mL of USP Alfuzosin System Suitability Mixture RS in Mobile phase
Sample solution: 0.40 mg/mL of Alfuzosin Hydrochloride in Mobile phase
Reference solution: 0.40 µg/mL of Alfuzosin Hydrochloride in Mobile phase from the Sample solution

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability (Sample: System suitability solution)
Suitability requirements
Peak-to-valley ratio: The ratio of the height of the furamide analog peak to the height of the valley between the furamide analog peak and alfuzosin is NLT 5.

Analysis Samples: Sample solution and Reference solution
Acceptance criteria: See Table. [Note—Disregard any peak with an area less than 0.05%.]

Name	RRT	Acceptance criteria - NMT (%)
Deacylated Alfuzosin[a]	0.5	0.2
Alfuzosin	1.0	-
Furamide analog[b]	1.2	-[c]
Any other individual, unidentified impurity	-	0.10
Total impurities	-	0.30

[a] N^2-(3-Aminopropyl)-6,7-dimethoxy-N^2-methylquinazoline-2,4-diamine.

[b] N-{3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl}furan-2-carboxamide.

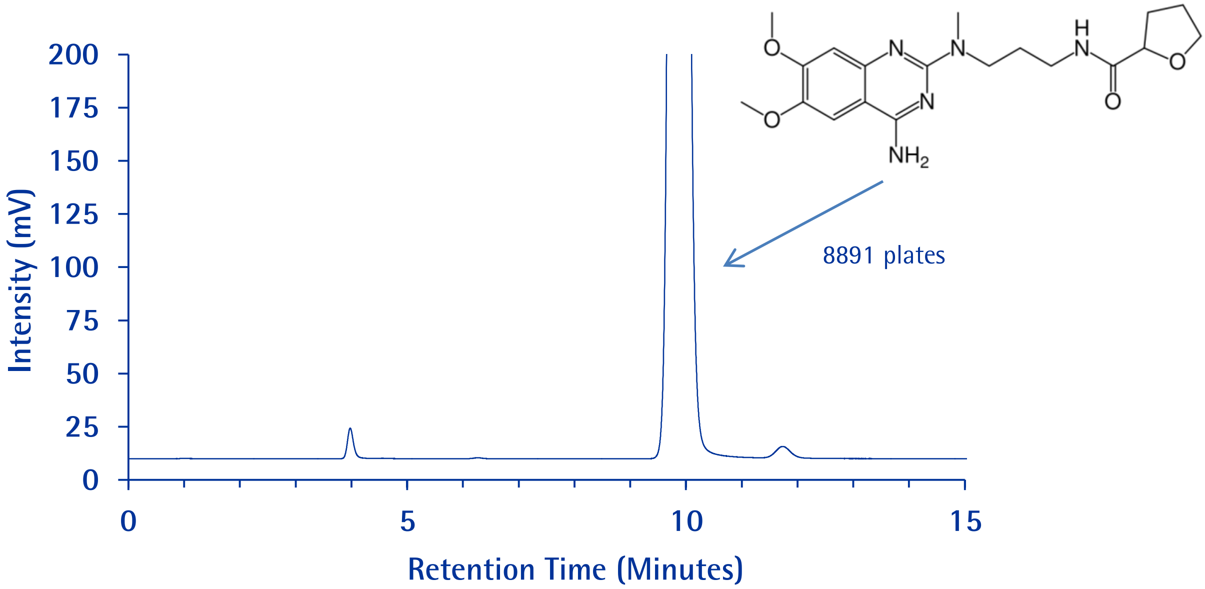
[c] Furamide analog, a component of USP Alfuzosin System Suitability Mixture RS, is not a specified impurity.

Alfuzosin and Related Substances (USP)

Particle packed column RP-18 endcapped

Chromatographic Conditions

Column:	Particle packed column, RP-18 endcapped, 150x4.6 mm
Injection:	10 µL
Detection:	UV 254 nm
Cell:	10 µL
Flow Rate:	1.5 mL/min
Mobile Phase (v/v):	5 ml of perchloric acid in 900 mL Milli-Q water. Adjust pH to 3.5 with 2M NaOH. Diluter to 1000 mL with water. Mix buffer, acetonitrile & THF 80:20:1 (v/v)
Temperature:	Ambient
Diluent	Mobile phase
Sample:	400 ppm (0.4 mg/mL) Alfuzozin RS in mobile phase
Pressure Drop:	150 Bar (2175 psi)



Chromatographic Data

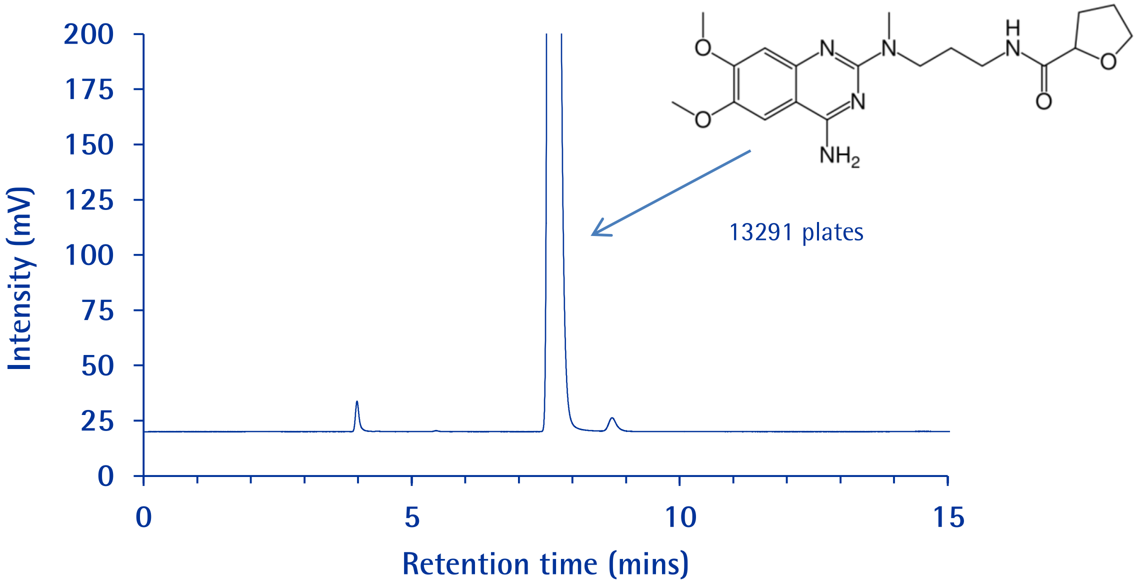
No	Compound	RT (min)	Resolution	Asymmetry (T_{USP})	RRT
1	Deacylated Alfuzosin	4.0	-	1.2	0.4
2	Alfuzosin	9.9	-	1.1	1.0
3	Furamide analog	11.7	4.1	1.1	1.2

Alfuzosin and Related Substances (USP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	10 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	5 ml of perchloric acid in 900 mL Milli-Q water. Adjust pH to 3.5 with 2M NaOH. Diluter to 1000 mL with water. Mix buffer, acetonitrile and THF 80:20:1 (v/v)	
Temperature:	25°C	
Sample preparation:	Dissolve 40.0 mg of the sample in 100 mL of mobile phase. 0.4 mg/mL (400 ppm) of USP Alfuzosin System Suitability Mixture RS in Mobile phase	
Reference solution:	0.40 µg/mL of Alfuzosin Hydrochloride in Mobile phase from the Sample solution	
Pressure Drop:	69 Bar (994 psi)	



Chromatographic Data

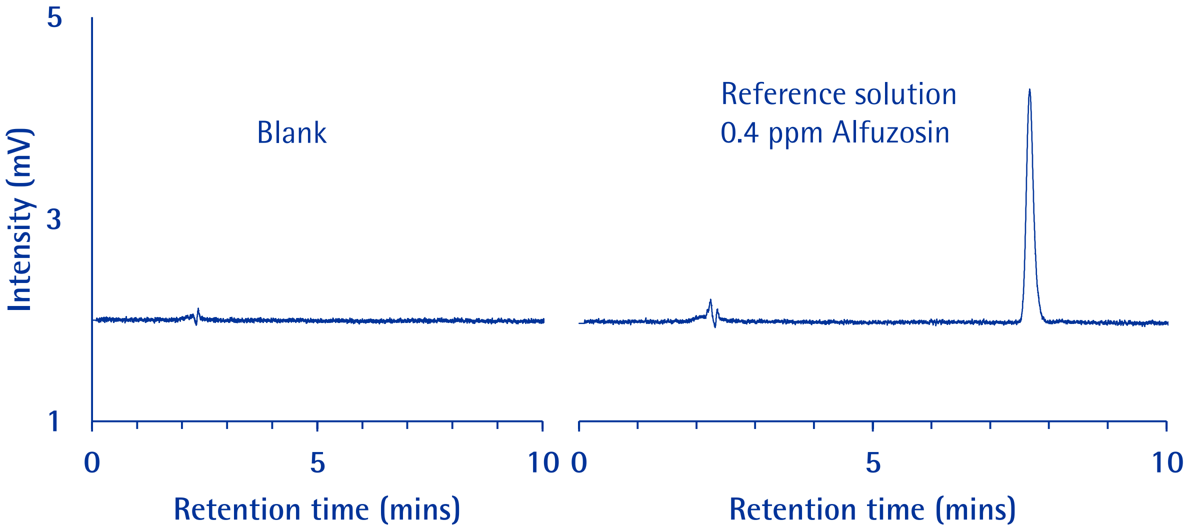
	Compound	RT (min)	RRT	TUSP	Resolution	Peak-to-valley ratio
1	Deacylated Alfuzosin	4.0	0.5	-	-	
2	Alfuzosin	7.6	1.0	1.2	-	
3	Furamide analog	8.8	1.2	-	4.3	22.0

Alfuzosin and Related Substances (USP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP18e 150x4.6 mm	1.52023.0001
Injection:	10 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	**1.0 mL/min**	
Mobile Phase:	5 ml of perchloric acid in 900 mL Milli-Q water. Adjust pH to 3.5 with 2M NaOH. Diluter to 1000 mL with water. Mix buffer, acetonitrile AND THF 80:20:1 (v/v)	
Temperature:	25°C	
Sample preparation:	Dissolve 40.0 mg of the sample in 100 mL of mobile phase. 0.4 mg/mL (400 ppm) of USP Alfuzosin System Suitability Mixture RS in Mobile phase	
Reference solution:	0.40 µg/mL of Alfuzosin Hydrochloride in Mobile phase from the Sample solution	
Pressure Drop:	**69 Bar (994 psi)**	



Chromatographic Data

No.	Compound	Retention Time (min)	Tailing factor	Theoretical plates
1	Alfuzosin	7.6	1.2	18875

Method Transfer – II

The second approach is to set-up the monograph method with a different type of column geometry than specified in the monograph text. Herein exemplified with Sildenafil citrate for which both the current assay and the organic impurities (related substances or impurity profile) method have been established, experimental details are given on page 28-29.

The monograph specify use of a 150x3.9 mm column with L1 (RP-18) packing having 5 µm particles operating at 1.0 mL/min. Flow rate changes can be made by using the equation on page 15 or it can be adjusted by ±50% (isocratic only). In this example, first a 150x4.6 mm monolithic column was used providing 39% larger column volume (2.5 instead of 1.8 mL) and the flow rate was therefore raised to 1.5 mL/min to start. Thereafter also a shorter monolithic column (100x4.6) was used with the flow rate given in the monograph, see data on page 33.

All performance criteria were fulfilled.

- **tailing factor should be NMT 1.5 (assay)**
- **the relative retention times for sildenafil, sildenafil N-oxide, and sildenafil related compound A are about 1.0, 1.2, and 1.7, respectively.**
- **Resolution: NLT 2.5 between sildenafil N-oxide and sildenafil**
- **Signal-to-noise ratio: NLT 10, Sensitivity solution**

The following benefits can be attained:

a) The method runs faster, either with the same length of column and higher flow rate, or a shorter column with original flow rate. This is especially important in the organic impurities/related substances method where t*he chromatograph should run for 3 times the retention time of sildenafil*.

b) High sensitivity - a combination of pure water, reagents, and solvents to make the mobile phase and the column, illustrated with the good S/N obtained for the sensitivity solution.

c) Low backpressure (<100 bar with both method alternatives) = less stress on HPLC system

Sildenafil Citrate (USP38-NF33)

Monograph method for Sildenafil Citrate – Assay and Organic Impurities

(USP38–NF33 Page 5292 - Pharmacopeial Forum: Volume No. 37(3))

Assay:

Mobile phase: Buffer, methanol, and acetonitrile (58:25:17)

Buffer: Dilute 7 mL of triethylamine with water to 1 L. Stir, and adjust with phosphoric acid to a pH of 3.0 ± 0.1.

Standard solution: 0.028 mg/mL of USP Sildenafil Citrate RS in Mobile phase
Sample solution: 0.028 mg/mL of Sildenafil Citrate in Mobile phase

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 290 nm
Column: 3.9-mm × 15-cm; 5-µm packing L1
Column temperature: 30°C
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability (Sample: Standard solution)
Suitability requirements **Tailing factor: NMT 1.5**
Relative standard deviation: NMT 0.85% for six replicate injections

Organic Impurities

Buffer, Mobile phase, and Chromatographic system: Proceed as directed in the Assay, except to *run the chromatograph for 3 times the retention time of sildenafil.*

Identification solution: 7.5 µg/mL of USP Sildenafil Related Compound A RS in Mobile phase

System suitability solution: Dissolve 70 mg of Sildenafil Citrate in 1 mL of a solution of hydrogen peroxide and anhydrous formic acid (2:1). Allow to stand for at least 10 min to generate sildenafil N-oxide, and then dilute with Mobile phase to 250 mL.

Sample solution: 0.7 mg/mL of Sildenafil Citrate in Mobile phase

Diluted sample solution: 1.4 µg/mL of sildenafil citrate in Mobile phase from the Sample solution

Sensitivity solution: 0.35 µg/mL of sildenafil citrate in Mobile phase from the Diluted sample solution

Sildenafil Citrate (USP38-NF33)

System suitability

Samples: Diluted sample solution, Sensitivity solution, and System suitability solution
[Note—The relative retention times for sildenafil, sildenafil N-oxide, and sildenafil related compound A are about 1.0, 1.2, and 1.7, respectively.]

Suitability requirements
Resolution: NLT 2.5 between sildenafil N-oxide and sildenafil, System suitability solution

Tailing factor: NMT 1.5 for the sildenafil peak, Diluted sample solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis - Acceptance criteria

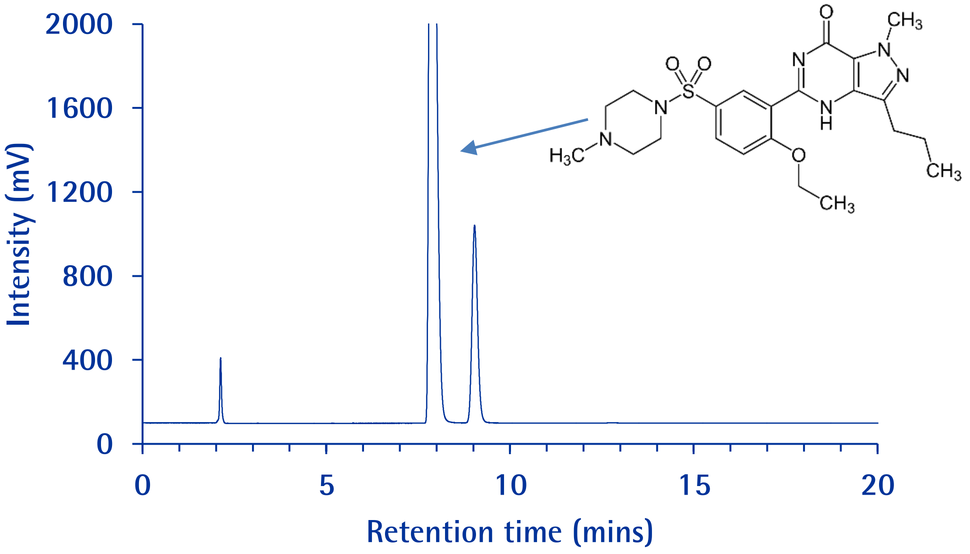
Sildenafil related compound A: NMT 0.3%
Any other unspecified individual impurity: NMT 0.10%
Total unspecified impurities: NMT 0.3%
Total impurities: NMT 0.5%. Disregard any peak less than 0.05%.

Sildenafil Citrate (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 150x4.6 mm	1.52023.0001
Injection:	20 µL	
Detection:	UV 290 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Buffer:	Dilute 7 mL of triethylamine with water to 1 L. Stir, and adjust with phosphoric acid to a pH of 3.0 ± 0.1.	
Mobile Phase:	Buffer, methanol, and acetonitrile (58:25:17)	
Temperature:	30°C	
Test solution:	0.7 mg/mL of Sildenafil Citrate in Mobile phase	
Resolution solution:	Dissolve 70 mg of Sildenafil Citrate in 1 mL of a solution of hydrogen peroxide and anhydrous formic acid (2:1). Allow to stand for at least 10 min to generate sildenafil N-oxide, and then dilute with Mobile phase to 250 mL.	
Pressure Drop:	80 bar (1160 psi)	

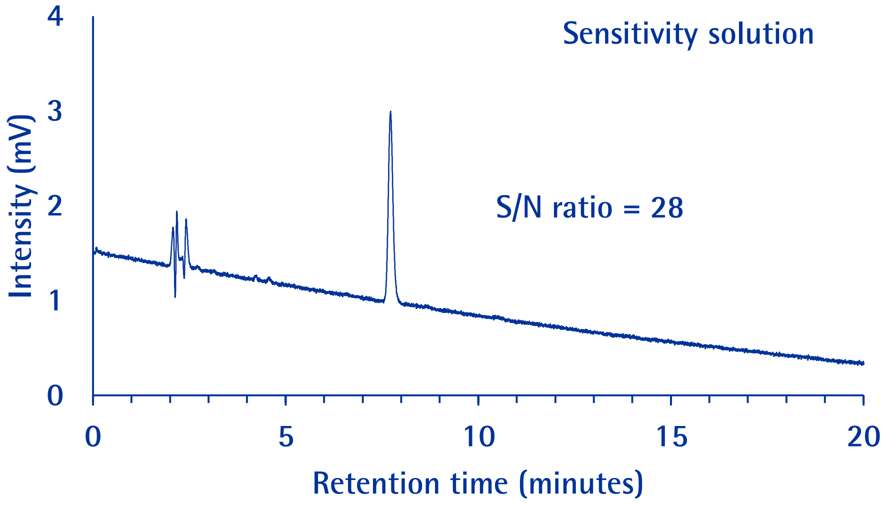


Chromatographic Data : Resolution solution

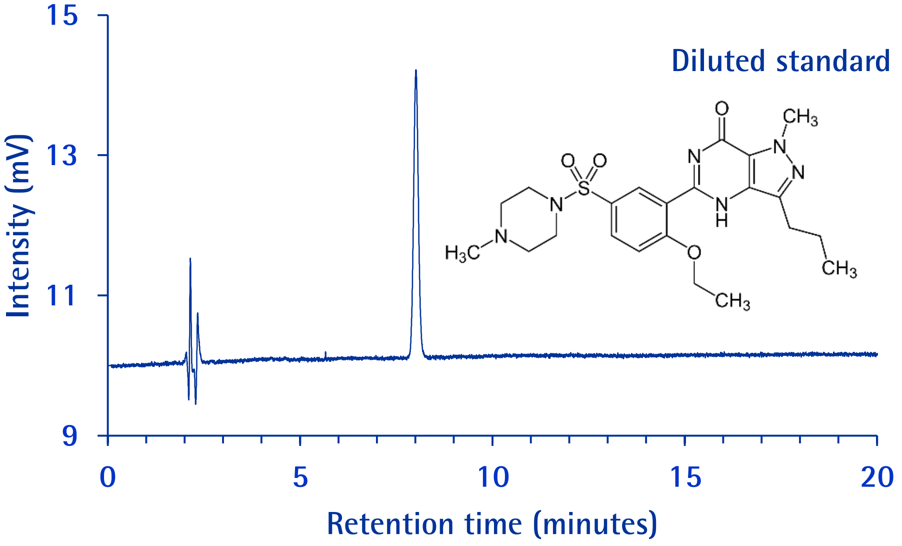
No.	Compound	RT (min)	RRT	Resolution	RRT
1	Sildenafil	7.9	-	0.0	1.0
2	Sildenafil N-oxide	9.1	1.2	4.0	1.2

Sildenafil Citrate (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped



Sensitivity solution: 0.35 µg/mL of sildenafil citrate in mobile phase from the diluted sample solution



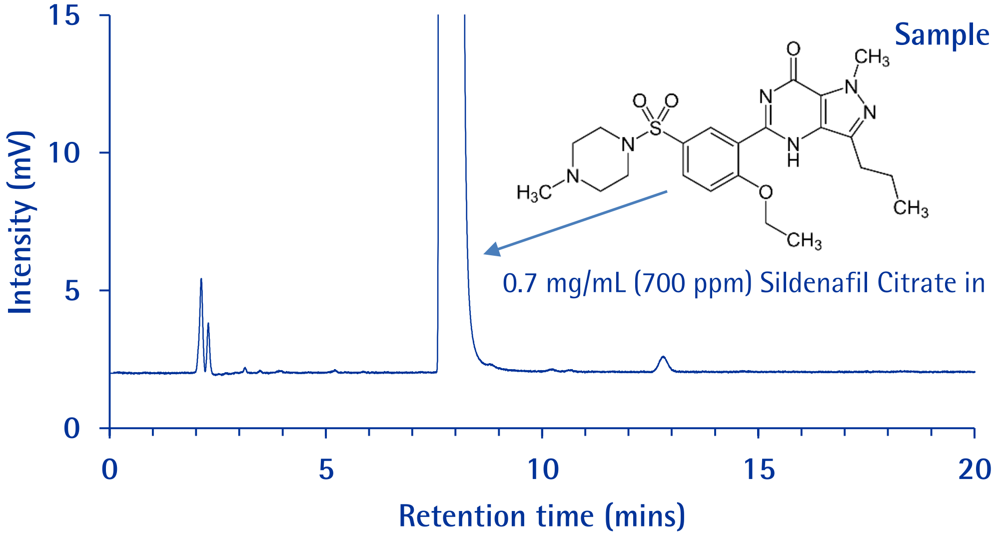
Diluted sample solution: 1.4 µg/mL of sildenafil citrate in Mobile phase from the Sample solution

Chromatographic Data (Diluted standard)

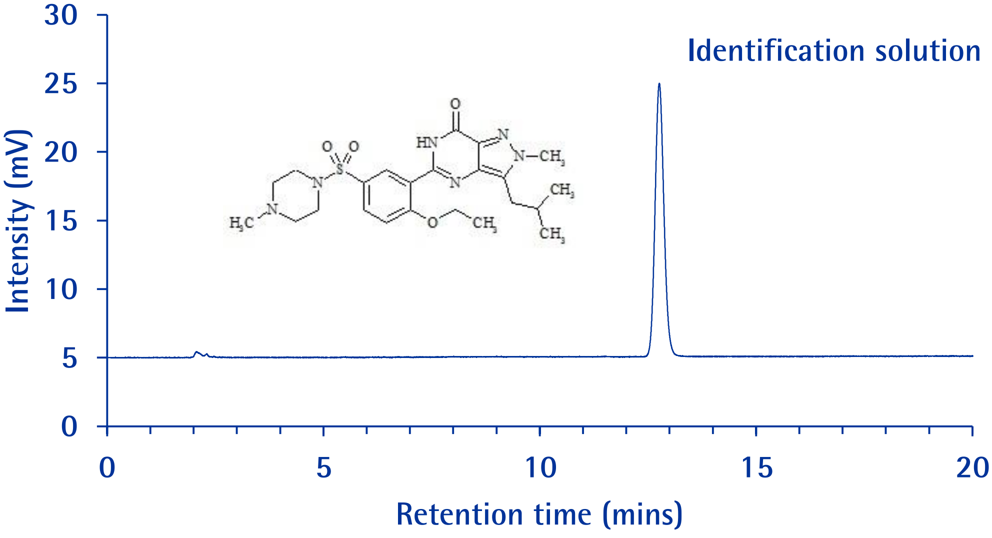
No.	Compound	Retention Time (min)	Tailing factor	Theoretical plates
1	Sildenafil	8.0	1.0	19695

Sildenafil Citrate (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped



0.7 mg/mL (700 ppm) Sildenafil Citrate in Mobile phase



Identification solution: 7.5 µg/mL of USP Sildenafil Related Compound A RS in Mobile phase

Chromatographic Data (Sample solution)

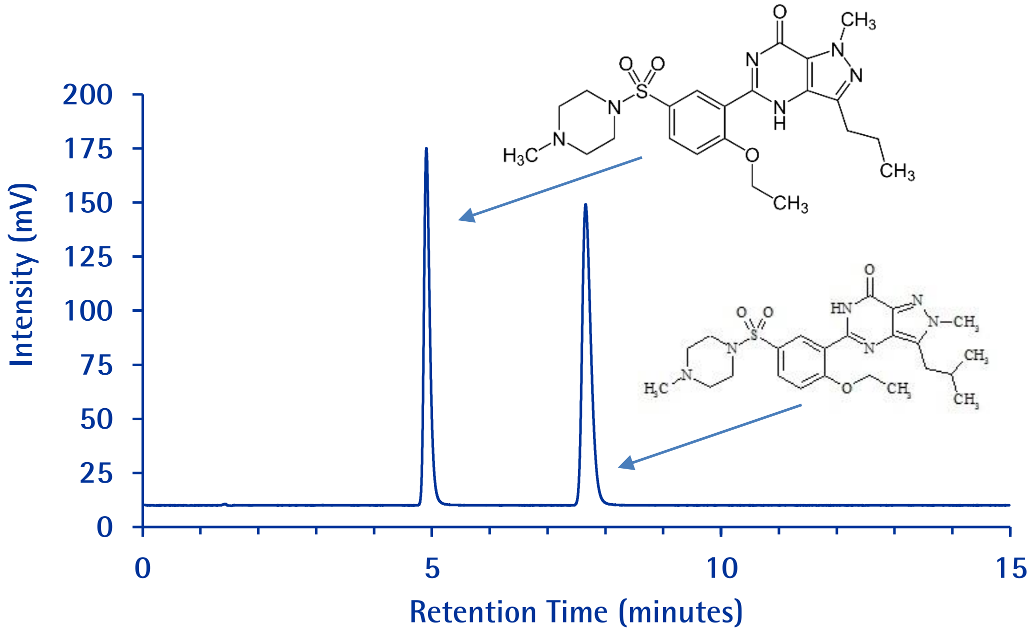
No.	Compound	Retention Time (min)	Tailing factor	RRT
1	Sildenafil	7.9	1.5	1.0
2	Sildenafil Related Substance A	12.8	1.0	1.63

Sildenafil Citrate (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV 290 nm	
Cell:	13 µL	
Flow Rate:	1.0 mL/min	
Buffer:	Dilute 7 mL of triethylamine with water to 1mL. Adjust with phosphoric acid to a pH=3.0±0.1	
Mobile Phase:	Mix the Buffer, Methanol and Acetonitrile 58:25:17 (v/v)	
Temperature:	30°C	
Diluent	Mobile phase	
Sample:	Sildenafil Citrate RS 28 ppm, Sildenafil Related Substance A 28ppm	
Pressure Drop:	67 Bar (971.5 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	Retention factor K°	Asymmetry
1	t0	1.4	-	-
2	Sildenafil Citrate RS	4.9	2.5	1.3
3	Sildenafil Related Substance A	7.6	4.4	1.4

Method Transfer - III

The third approach is to set-up a monograph method, push the method towards the limits of its allowed performance criteria. The current Albuterol Tablets assay method is used as an example. The experimental details are given on page 35.

The Albuterol Tablets monograph has very generous suitability criteria and with a proposed 150x4.6 mm column having L1 (RP-18) packing operating at 1.5 mL/min. No particle size is mentioned, thus to scale the method using the equation on page 15 one need to use largest particle size in the packing definition (10 um), or alternatively it can be adjusted by ±50%.

Pages 35-39 illustrate the method performance when changing the column length from 150-50 mm and the mobile phase composition (± 30% relative, or ± 10% absolute whichever is smaller).

The system suitabilitiy criteria were fulfilled in all experiments.

- Column efficiency: NLT 800 theoretical plates
- Tailing factor: NMT 2.5
- Relative standard deviation: NMT 2.0%

The method can run faster and would be approved, using the shortest column, with reduced injection volume and with an altered mobile phase composition. However, both the method sensitivity (LOQ is close to the sample concentration) and column efficiency is strongly affected.

Considering method robustness the method would thus benefit from a longer column and either the 100 or 150 mm Chromolith® HighResolution RP-18 endcapped can be used and meet .

Albuterol Tablets – Assay (USP38-NF33)

Monograph method for Albuterol Tablets

(USP38–NF33 Page 2072 - Pharmacopeial Forum: Volume No. 31(3) Page 726)

Assay:

Solution A: 10 mL/L of glacial acetic acid in water
Solution B: 1.13 g of sodium 1-hexanesulfonate in 1200 mL of water. Add 12 mL of glacial acetic acid.
Diluent: Methanol and water (40:60)
Mobile phase: Methanol and Solution B (40:60)

Standard stock solution: 0.12 mg/mL of USP Albuterol Sulfate RS, prepared as follows.
Transfer USP Albuterol Sulfate RS to a suitable volumetric flask, and add a volume of Solution A corresponding to 60% of the flask volume. Sonicate for 5 min, and dilute with methanol to volume.

Standard solution: 0.03 mg/mL of USP Albuterol Sulfate RS in Diluent, from Standard stock solution

Sample solution: Nominally 0.025 mg/mL of albuterol, prepared as follows.
Transfer a number of whole Tablets, equivalent to 50 mg of albuterol, to a suitable volumetric flask.
Add 60% of the flask volume of Solution A, shake by mechanical means for 45 min, sonicate for 10 min, allow to cool to room temperature, and dilute with methanol to volume.
Pass through a suitable filter of 0.45-µm or finer pore size.

Chromatographic system (See Chromatography 621, System Suitability.)

Detector: UV 276 nm
Column: 4.6-mm × 15-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL

System suitability (Sample: Standard solution)

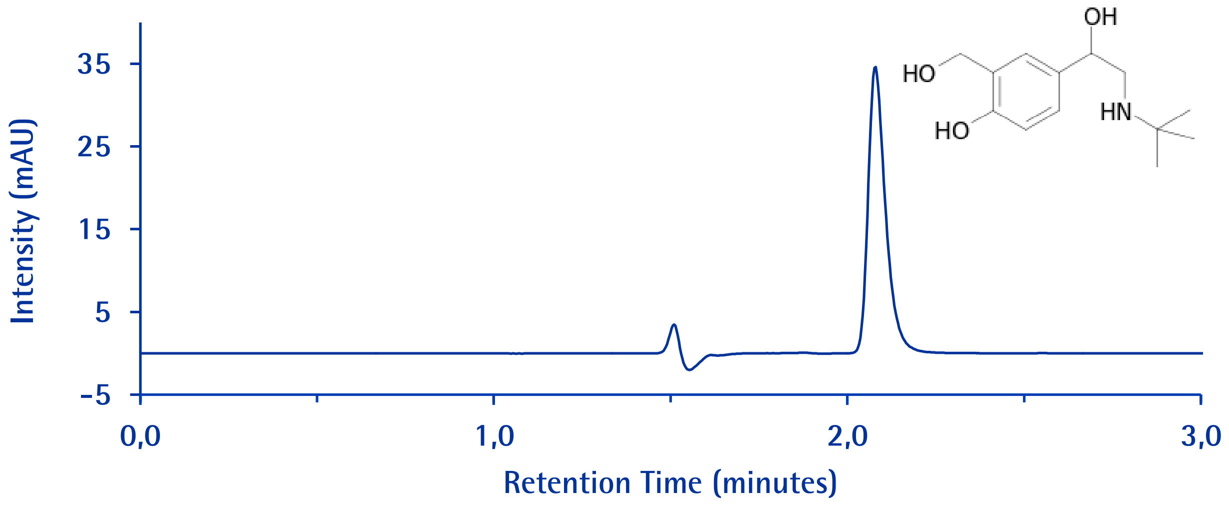
Suitability requirements	Column efficiency: NLT 800 theoretical plates Tailing factor: NMT 2.5 Relative standard deviation: NMT 2.0%

Albuterol Tablets – Assay (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	25 µL	
Detection:	UV, 276 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Solution A:	10mL/L of glacial acetic acid in water	
Solution B:	1.13g of sodium 1-Hexanesulfate in 1200mL water, add 12mL of glacial acetic acid	
Mobile phase:	Solution A and B 40:60 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol and Water 40:60 (v/v)	
Sample:	Albuterol RS 30 µg/mL in diluent	
Sample:	30 µg/mL of Albuterol Sulfate RS in Diluent from Standard stock solution	
Stock Solution:	120 µg/mL of Albuterol Sulfate RS prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask, and add a volumne of Solution A corresponding to 60% of the flask volume. Sonicate for 5 minutes, and dilute with Methanol to volume.	
Pressure Drop:	138 Bar (2001 psi)	



Chromatographic Data

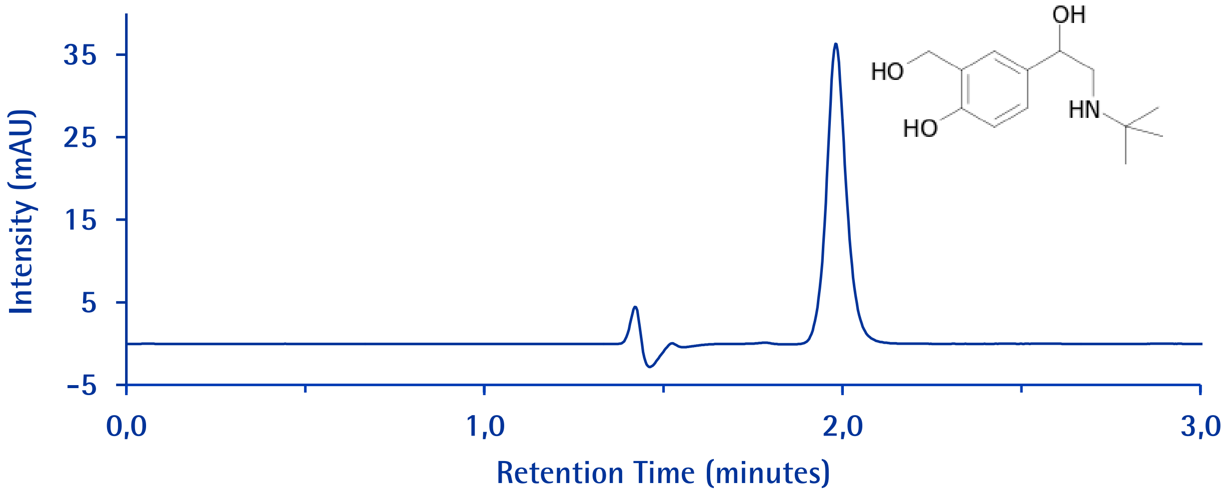
No	Compound	Retention Time (min)	Capacity factor	RRT	Plates$_{(USP)}$	T_{USP}
1	T_0 (void volume)	1.5	-	-	-	-
2	Albuterol RS	2.1	0.4	1.4	8939	1.4

Albuterol Tablets – Faster Assay (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	25 µL	
Detection:	UV, 276 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Solution A:	10mL/L of glacial acetic acid in water	
Solution B:	1.13g of sodium 1-Hexanesulfate in 1200mL water, add 12mL of glacial acetic acid	
Mobile phase:	Solution A and B 36:64 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol and Water 40:60 (v/v)	
Sample:	Albuterol RS 30 µg/mL in diluent	
Sample:	30 µg/mL of Albuterol Sulfate RS in Diluent from Standard stock solution	
Stock Solution:	120 µg/mL of Albuterol Sulfate RS prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask, and add a volumne of Solution A corresponding to 60% of the flask volume. Sonicate for 5 minutes, and dilute with Methanol to volume.	
Pressure Drop:	114 Bar (1653 psi)	



Chromatographic Data

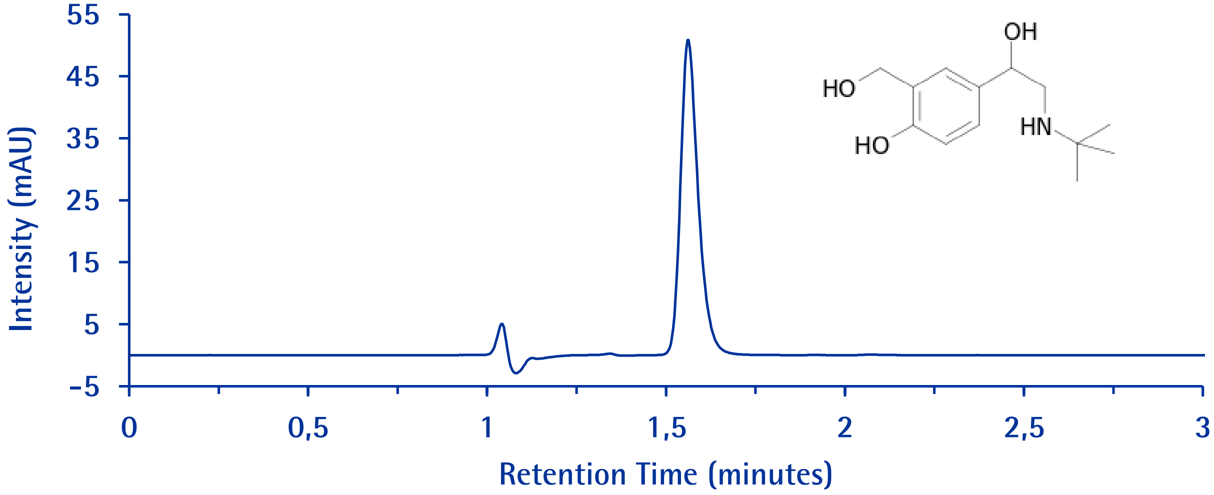
No	Compound	Retention Time (min)	Capacity factor	RRT	Plates$_{(USP)}$	T_{USP}
1	T_0 (void volume)	1.4	-	-	-	-
2	Albuterol RS	2.0	0.4	1.4	9222	1.3

Albuterol Tablets – Faster Assay (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	17 µL	
Detection:	UV, 276 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Solution A:	10mL/L of glacial acetic acid in water	
Solution B:	1.13g of sodium 1-Hexanesulfate in 1200mL water, add 12mL of glacial acetic acid	
Mobile phase:	Solution A and B 36:64 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol and Water 40:60 (v/v)	
Sample:	Albuterol RS 30 µg/mL in diluent	
Sample:	30 µg/mL of Albuterol Sulfate RS in Diluent from Standard stock solution	
Stock Solution:	120 µg/mL of Albuterol Sulfate RS prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask, and add a volumne of Solution A corresponding to 60% of the flask volume. Sonicate for 5 minutes, and dilute with Methanol to volume.	
Pressure Drop:	108 Bar (1553 psi)	



Chromatographic Data

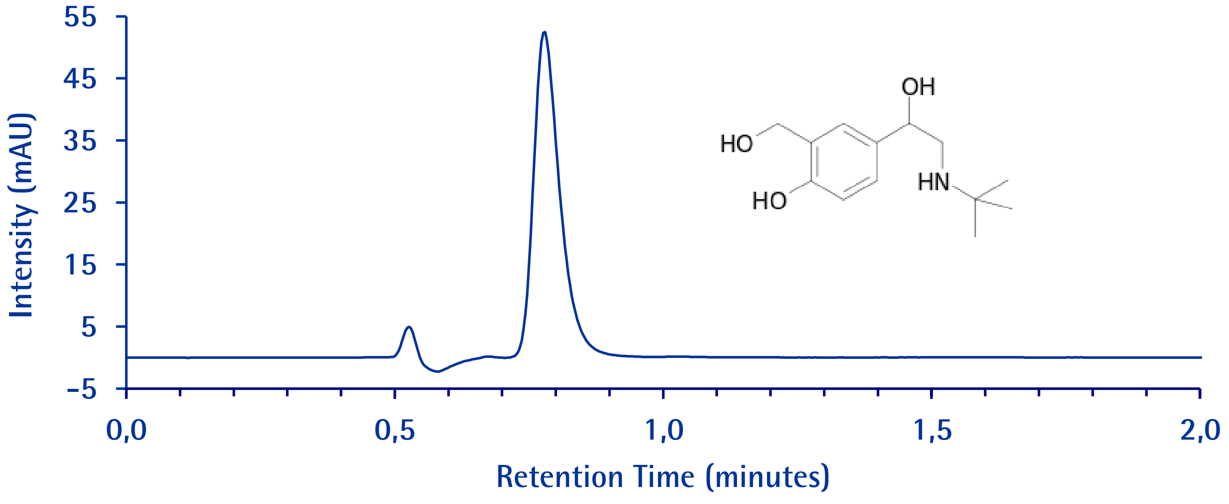
No	Compound	Retention Time (min)	Capacity factor	RRT	Plates$_{(USP)}$	T_{USP}
1	T_0 (void volume)	1.0	-	-	-	-
2	Albuterol RS	1.6	0.5	1.5	5012	1.3

Albuterol Tablets – Faster Assay (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 50x4.6 mm	1.52021.0001
Injection:	8 µL	
Detection:	UV, 276 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Solution A:	10mL/L of glacial acetic acid in water	
Solution B:	1.13g of sodium 1-Hexanesulfate in 1200mL water, add 12mL of glacial acetic acid	
Mobile phase:	Solution A and B 36:64 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol and Water 40:60 (v/v)	
Sample:	Albuterol RS 30 µg/mL in diluent	
Sample:	30 µg/mL of Albuterol Sulfate RS in Diluent from Standard stock solution	
Stock Solution:	120 µg/mL of Albuterol Sulfate RS prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask, and add a volumne of Solution A corresponding to 60% of the flask volume. Sonicate for 5 minutes, and dilute with Methanol to volume.	
Pressure Drop:	82 Bar (1189 psi)	



Chromatographic Data

No	Compound	Retention Time (min)	Capacity factor	RRT	$\text{Plates}_{(USP)}$	T_{USP}
1	T_0 (void volume)	0.5	-	-	-	-
2	Albuterol RS	0.8	0.4	1.4	1262	1.4

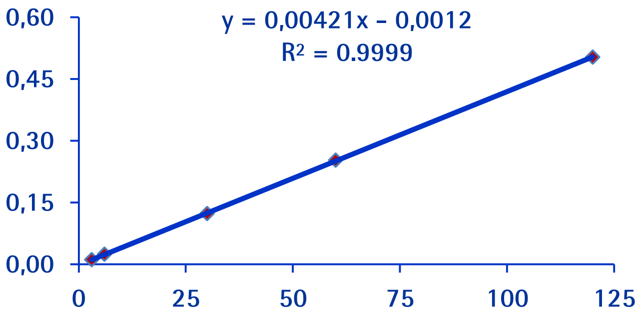
Albuterol Tablets – Faster Assay (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Linearity: Analysis of repeated injections (n=5) of standard solutions in the calibration range 3-120 ppm (µg/mL).

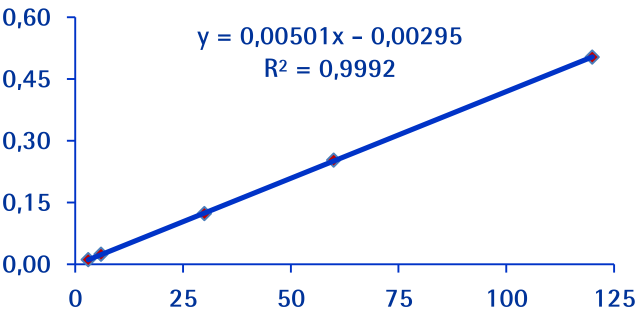
150x4.6 mm column

40:60 mobile phase solution A and B



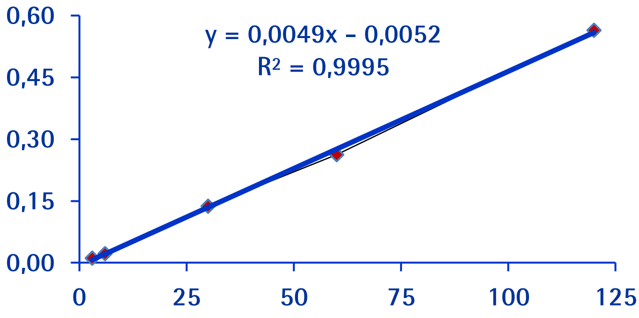
LOQ (ppm)	LOD (ppm)
1.1	3.4

36:64 mobile phase solution A and B



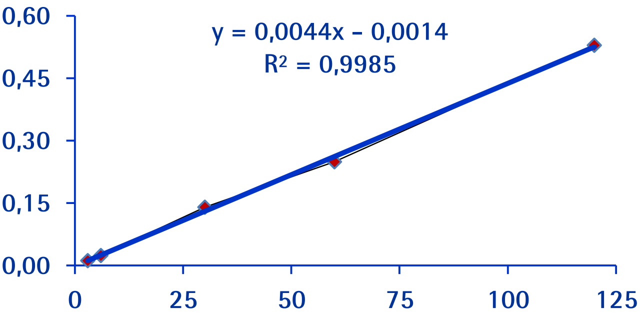
LOQ (ppm)	LOD (ppm)
2.8	8.4

100x4.6 mm column



LOQ (ppm)	LOD (ppm)
4.8	14.4

50x4.6 mm column



LOQ (ppm)	LOD (ppm)
7.3	22

Method Transfer – IV

The fourth example illustrate the possibilities within certain HPLC monograph methods that specify different chemistries for the same method. The current methods for Esomeprazole Magnesium serve as example where both L7 and L1 packings can be used for both assay and impurities. The complete method details can be found elsewhere in previous compilations), see the link for download (complete monograph methods & solutions for dissolution testing)

http://www.merckmillipore.com/application-collections

The Esomeprazole Magnesium monograph specify the use of either 125x4.0 mm or a 150x4.6 mm; 5 µm packing L7 (RP-8). Alternatively, a 150x3.9mm column that contains 4 µm packing L1 may be used. Pages 42-43 illustrate that the system suitability can be met with three different type of column geometries and with different packing.

(Assay)	- Column efficiency: NLT 2000 theoretical plates
(impurities)	- Resolution NLT 3 between omeprazole related compound A and omeprazole
	- RRT, 0.45 (Omeprazole N-oxide), 0.8 (omeprazole related compound A) and 1.0 for omeprazole
	- record the chromatogram for at least 4.5X the omeprazole peak retention time

A fast method can save solvent and time. The quickest chromatogram is attained with the 2 µm particle packed column, but it also generate the highest backpressure. Special instrumentation is therefore required to handle the high pressures and more mechnical stress can be expected, leading to more maintenance.

Page 42 show an example how you can utilize a shorter column (100 mm) with L1 packing to reduce the run-time for the method. The L1 packing provides higher retention and therefore also more resolution between the omeprazole related compound A and the omeprazole peak. L1 is more hydrophobic than L7. Taking a slightly larger diameter (4.6 instead of 3.9 mm) it is possible to keep a comparable total tube volume (a 100x4.6 mm and 150x3.9 column have both roughly 1.7 mL column volume) and effectively promote high loadability which is important when running impurity profiling methods.

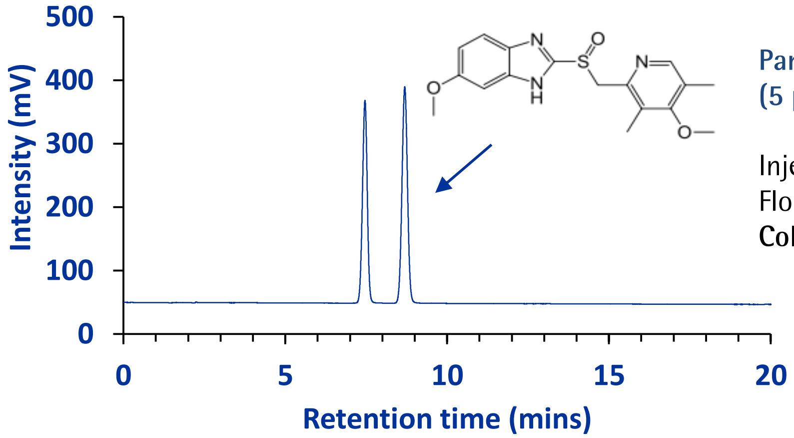
Esomeprazole Magnesium (USP38-NF33)

USP Esomeprazole Assay Method

Column: 125x4 mm or 150x4.6 mm column; 5 µm packing L7
Alternatively 150x3.9 mm column; 4 µm packing L1 may be used.

Flow rate: 0.8–1 mL/min

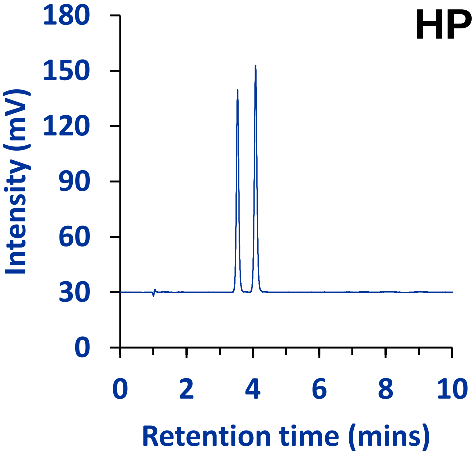
Injection size: 50 µL



Particulate Column RP-8 endcapped
(5 µm) 150x4.6 mm

Injection volume: 50 µL
Flow rate: 1.0 mL/min
Column Pressure: 87 Bar (1261 psi)

Compound	RT (min)	Plates	Resolution	RRT
Omeprazole Related Substance A	7.46	11978	-	0.85
Omeprazole	8.69	12271	4.2	1.00



HPLC to UHPLC

$F_2 = 1.0 \times [(2.1^2 \times 5)/(4.6^2 \times 2)] = 0.52$ mL/min

Particulate Column RP-8 endcapped
(2 µm) 100x2.1 mm
Injection volume: 5 µL (10x reduction)
Flow rate: 0.3 mL/min
(reduced to get same resolution and plates)

Column Pressure: 300 Bar (4500 psi)

Compound	RT (min)	Plates	Resolution	RRT
Omeprazole Related Substance A	3.5	11746	-	0.85
Omeprazole	4.1	12928	4.2	1.00

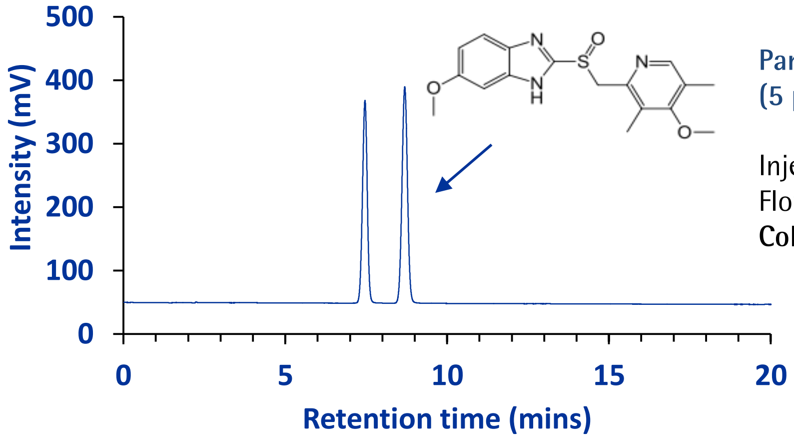
Esomeprazole Magnesium (USP38-NF33)

USP Esomeprazole Assay Method

Column: 125x4 mm or 150x4.6 mm column; 5 µm packing L7
Alternatively 150x3.9 mm column; 4 µm packing L1 may be used.

Flow rate: 0.8–1 mL/min

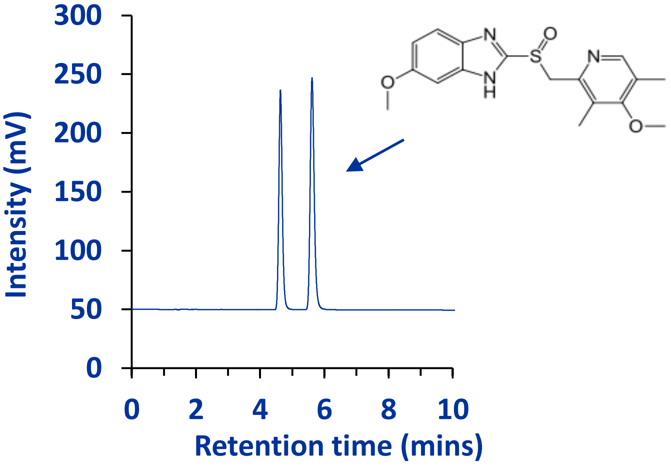
Injection size: 50 µL



Particulate Column RP-8 endcapped (5 µm) 150x4.6 mm

Injection volume: 50 µL
Flow rate: 1.0 mL/min
Column Pressure: 87 Bar (1261 psi)

Compound	RT (min)	Plates	Resolution	RRT
Omeprazole Related Substance A	7.46	11978	-	0.85
Omeprazole	8.69	12271	4.2	1.00



Chromolith® HighResolution RP-18e 100x4.6 mm

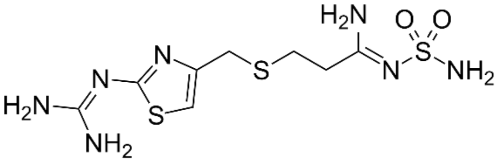
Injection volume: 20 µL (2.5 times decrease)
Flow rate: 1.0 mL/min

Column Pressure: 50 Bar (725 psi)

Higher retention and improved resolution

Compound	RT (min)	Plates	Resolution	RRT
Omeprazole Related Substance A	4.6	9998	-	0.82
Omeprazole	5.6	10925	4.7	1.00

Famotidine (USP38-NF33)



Famotidine is a histamine H2-receptor antagonist that inhibits stomach acid production.
It is commonly used in the treatment of peptic ulcer disease and gastroesophageal reflux disease.

Common commercial brand names: Pepcid

Famotidine was developed by Yamanouchi Pharmaceutical Co and it was first marketed in 1981, and licensed in the mid-80s by Merck & Co.

Generic preparations became available in 2001, for instance Fluxid or Quamatel.

There are different HPLC methods for famotidine, famotidine injection, famotidine tablets etc. This compilation focus on the famotidine tablets monograph which is listed in the USP monograph modernization project.

http://www.usp.org/usp-nf/development-process/priority-new-monographs/list/

The current USP method had to be modifed within allowed changes (see chapter 621) :

- Mobile phase was made weaker to allow more retentivity for the famotidine peak
- flow rate was increased to compress the eluting peaks for better peak symmetry

The modified method passes all system suitability criteria.

Famotidine Tablets (USP38-NF33)

(USP38–NF33 Page 3441 - Pharmacopeial Forum: Volume No. 37(3)

Buffer: Dissolve 13.6 g of sodium acetate trihydrate in 750 mL of water.
Add 1 mL of triethylamine, adjust with glacial acetic acid to a pH of 6.0, dilute with water to 1 L.

Mobile phase: Acetonitrile and Buffer (7:93)

Diluent: Dissolve 6.8 g of monobasic potassium phosphate in 750 mL of water, adjust with 1 M potassium hydroxide to a pH of 6.0, and dilute with water to 1 L.

System suitability stock solution:

- Transfer 10 mg of famotidine to a 50-mL volumetric flask.
- Add 1 mL of 0.1 N hydrochloric acid, heat at 80 for 30 min, and cool to room temperature.
- Add 2 mL of 0.1 N sodium hydroxide, heat at 80 for 30 min, cool to room temperature
- Neutralize by adding 1 mL of 0.1 N hydrochloric acid.
- Dilute with Diluent to volume.
- Transfer 10 mL of this solution to a separate 50-mL volumetric flask containing 5 mg of famotidine dissolved in 8 mL of methanol.
- Dilute with Diluent to volume.
- Transfer 25 mL of this solution to a 50-mL volumetric flask, and dilute with Diluent to volume. *[Note—This solution is stable for up to 1 month.]*

System suitability solution: Transfer 1–1.5 mL of the System suitability stock solution to a suitable container, add 1 drop of hydrogen peroxide solution, and mix well. [Note—Prepare fresh daily.]

Standard solution: Transfer 10 mg of USP Famotidine RS to a 100-mL volumetric flask, add 20 mL of methanol, and sonicate for 5 min. Dilute with Diluent to volume.

Sample solution: Transfer NLT 10 Tablets to a 1-L volumetric flask. Add 200 mL of Diluent, and swirl to erode the Tablets. Add 200 mL of methanol, and stir by mechanical means at 300 rpm for 1 h. Dilute with Diluent to volume, mix, and filter. Quantitatively dilute a portion of the clear filtrate with Diluent to obtain a solution containing about 0.1 mg/mL of famotidine.

Famotidine Tablets (USP38-NF33)

(USP38–NF33 Page 3441 - Pharmacopeial Forum: Volume No. 37(3)

Chromatographic system (See Chromatography 621, System Suitability.)

Detector: UV 275 nm
Column: 150x4.6 mm; packing L1 *(No particle size referred)*
Column temperature: 40°C
Flow rate: 1.4 mL/min

Injection size: 50 µL

System suitability
Samples: System suitability solution and Standard solution

Suitability requirements - Identify peaks using Table

Resolution: NLT 1.3 between the famotidine sulfamoyl propanamide and famotidine peaks, and
Resolution: NLT 1.3 between the famotidine and famotidine propanamide peaks,
(System suitability solution)

Capacity factor: NLT 2.0 for the famotidine peak (System suitability solution)

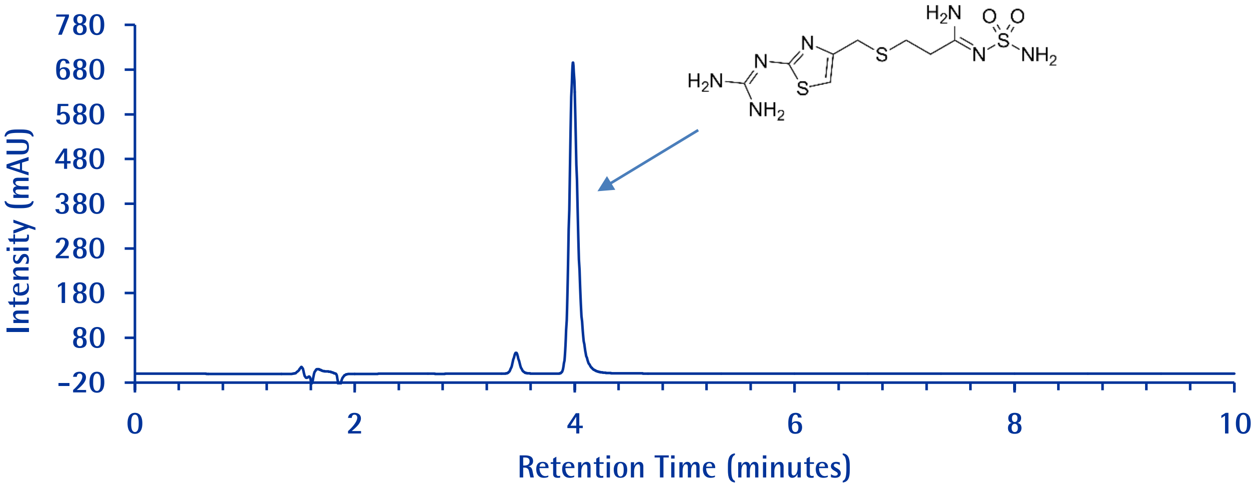
Name		RRT	RRF	Acceptance criteria (NMT %)
Famotidine sulfoxide		0.4	1.0	1.0
Famotidine propionic acid	(Related compound F)	0.7	1.0	0.5
Famotidine sulfamoyl propanamide	(Related compound C)	0.8	1.0	0.5
Famotidine		1.0	-	-
Famotidine propanamide	(Related compound D)	1.2	1.3	0.5
Total impurities		80	20	1.5

Famotidine Tablets – original (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	50 µL	
Detection:	UV, 275 nm	
Cell:	11 µL	
Flow Rate:	1.4 mL/min	
Buffer:	13.6 g sodium acetate trihydrate in 750 mL water Add 1 mL Triethylamine, adjust with glacial acetic acid to pH = 6.0, dilute with water to 1 L.	
Mobile phase:	Buffer and acetonitrile 93:7 (v/v)	
Temperature:	40 °C	
Diluent	Dissolve 6.8 g of monobasic potassium phosphate in 750 mL of water. Adjust with 1 M potassium hydroxide to pH = 6.0 and dilute with water to 1 L.	
Sample:	System suitability solution	
Pressure Drop:	48 bar (696 psi)	



Chromatographic Data

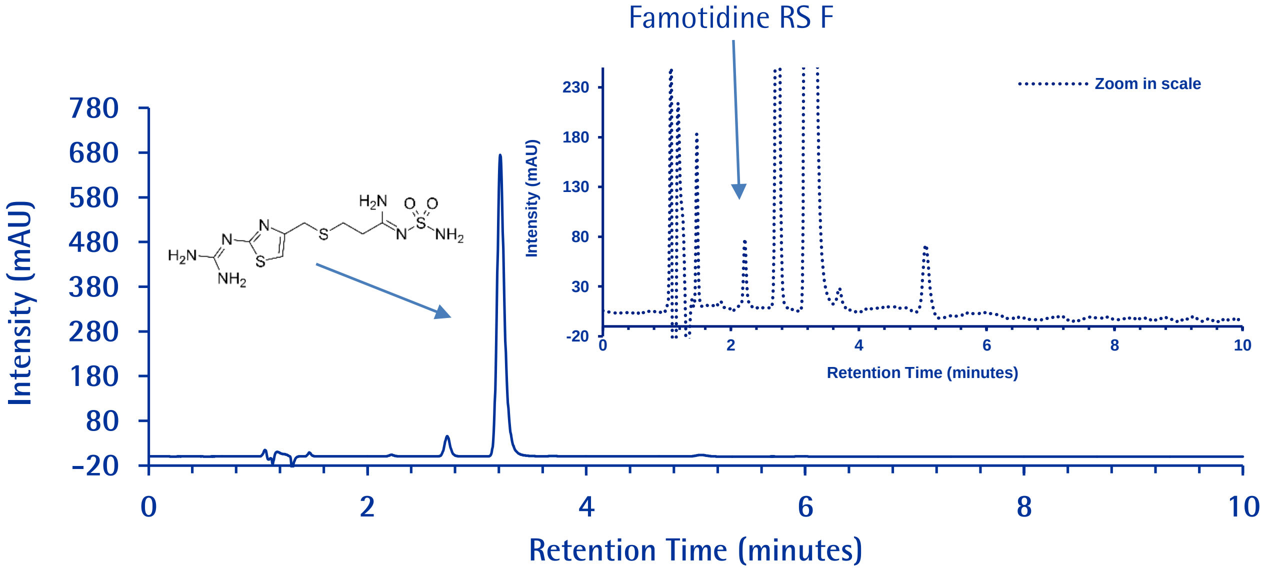
No	Compound	Retention Time (min)	Capacity factor	RRT	Resolution
1	T_0 (void volume)	1.5	-	-	-
2	Famotidine RS C	3.5		0.9	-
3	Famotidine	4.0	1.6	-	4.2
4	Famotidine RS D	4.5	-	1.1	4.2

Famotidine Tablets –modified (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	50 µL	
Detection:	UV, 275 nm	
Cell:	11 µL	
Flow Rate:	2.0 mL/min	
Buffer:	13.6 g sodium acetate trihydrate in 750 mL water Add 1 mL Triethylamine, adjust with glacial acetic acid to pH = 6.0, dilute with water to 1 L.	
Mobile phase:	Buffer and acetonitrile 93.7:6:3 (v/v)	
Temperature:	40 °C	
Diluent	Dissolve 6.8 g of monobasic potassium phosphate in 750 mL of water. Adjust with 1 M potassium hydroxide to pH = 6.0 and dilute with water to 1 L.	
Sample:	System suitability solution	
Pressure Drop:	79 bar (1146 psi)	



Chromatographic Data

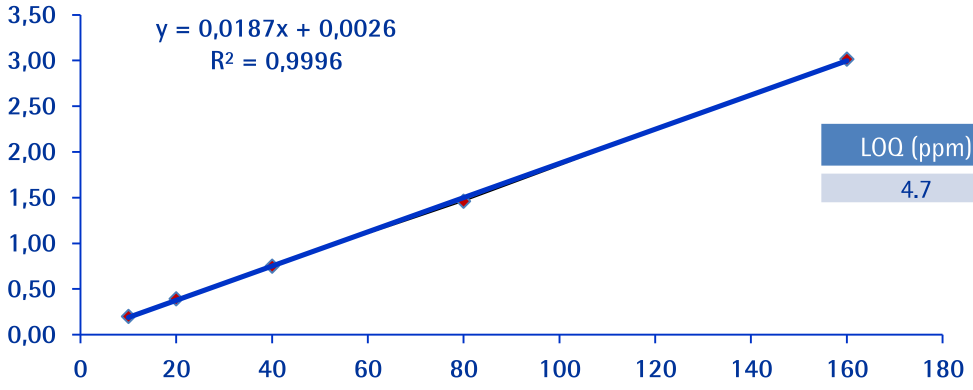
No	Compound	Retention Time (min)	Capacity factor	RRT	Resolution
1	T_0 (void volume)	1.1	-	-	-
2	Famotidine RS C	2.7		0.8	-
3	Famotidine	3.2	2.1	-	4.6
4	Famotidine RS D	3.7	-	1.2	4.0

Famotidine Tablets –modified (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

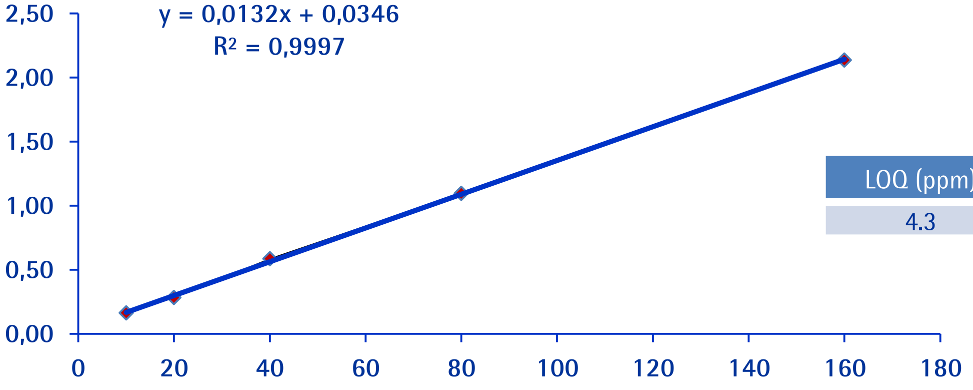
Linearity: Analysis of repeated injections (n=5) of standard solutions of famotidine in the calibration range 10-160 ppm (µg/mL).

93:7 mobile phase solution A and B
Flow rate: 1.4 mL/min



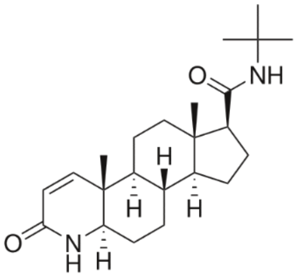
LOQ (ppm)	LOD (ppm)
4.7	14.1

93.7:6.3 mobile phase solution A and B
Flow rate: 2.0 mL/min



LOQ (ppm)	LOD (ppm)
4.3	13.2

Finasteride (USP38-NF33)



Finasteride is used for the treatment of benign prostatic hyperplasia (BPH) and male pattern baldness (MPB). It is a type II and type III 5α-reductase inhibitor; 5α-reductase, an enzyme, converts testosterone to dihydrotestosterone (DHT).

Common commercial brand names: Proscar, Propecia

Finasteride in the form of Proscar was introduced in 1992 by MSD (Merck Sharp & Dohme outside the United States and Canada and known as Merck & Co in North America).

There are two different monographs for finasteride (finasteride and finasteride tablets) and where this compilation focus on tablets monograph.

The current USP method had to be modifed within allowed changes (see chapter 621) :

- **Mobile phase was weakened to allow more retentivity for the finasteride peak to pass the capacity criteria of the method.**
- flow rate was increased to compress the eluting peaks for better peak symmetry
- The method was transferred to a shorter column

The modified method passes all system suitability criteria.

Finasteride Tablets (USP38-NF33)

(USP38–NF33 Page 3502 - Pharmacopeial Forum: Volume No. 32(6) Page 1681)

Mobile phase:
Prepare a filtered & degassed mixture of 2.5 mM phosphoric acid and acetonitrile (1:1).
Make adjustments if necessary (see System Suitability under Chromatography 621).

Diluting solution: Prepare a solution of acetonitrile and water (7:3).

Standard preparation: Dissolve an accurately weighed quantity of USP Finasteride RS in Diluting solution, and dilute quantitatively, and stepwise if necessary, with Diluting solution to obtain a solution having a known concentration of about 100 µg per mL (100 ppm).

Assay preparation: Weigh and finely powder not fewer than 20 Tablets. Transfer an amount of powder equivalent to about 10 mg of finasteride, accurately weighed, to a 100 mL volumetric flask, dissolve in and dilute with Diluting solution to volume, and mix.

Chromatographic system (see Chromatography 621):
The liquid chromatograph is equipped with a 240 nm detector and a 100x4.6 mm column that contains packing L1. The column temperature is maintained at 45 degrees Celcius. The flow rate is about 1.5 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure:

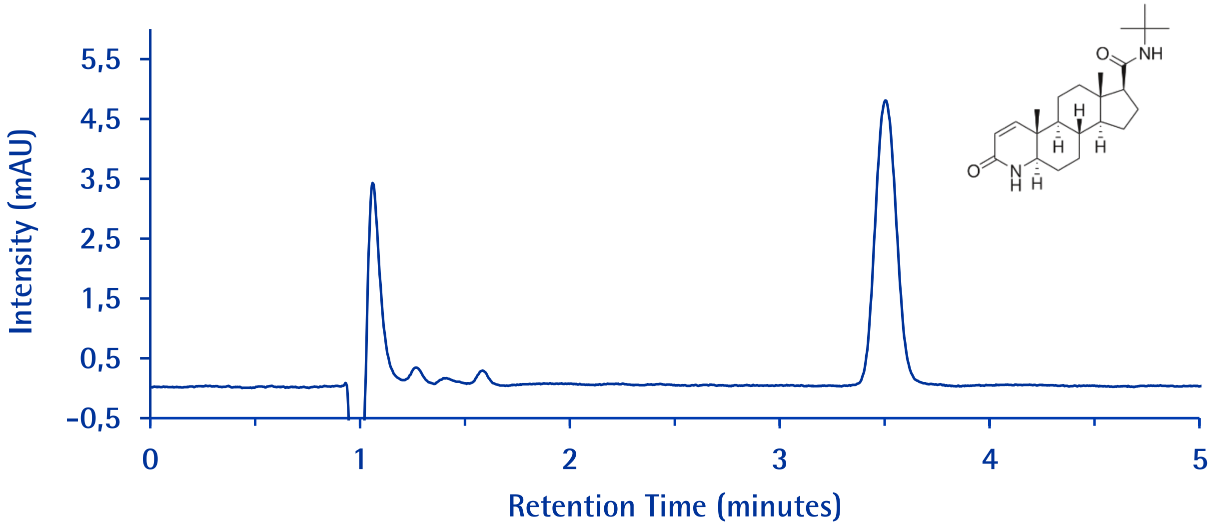
- the capacity factor, k, is not less than 2.0;
- the column efficiency is not less than 1000 theoretical plates;
- the tailing factor is not more than 2.0; and
- the relative standard deviation for replicate injections is not more than 2.0%.

Finasteride Tablets –modified 1 (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV, 240 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Acetonitrle and 2.5 mM phosphoric acid in Water 40:60 (v/v) (monograph text says 1:1)	
Temperature:	45 °C	
Diluent:	Acetonitrile and water 70:30 (v/v)	
Sample Solution:	Finasteride RS 100 µg/mL (100 ppm)	
Sample prep.:	Dissolve an accurately weighed quantity of USP Finasteride RS in Diluting solution, and dilute quantitatively, and stepwise if necessary, with Diluting solution to obtain a solution having a known concentration of about 100 µg per mL.	
Pressure Drop:	53 Bar (624 psi)	



Chromatographic Data

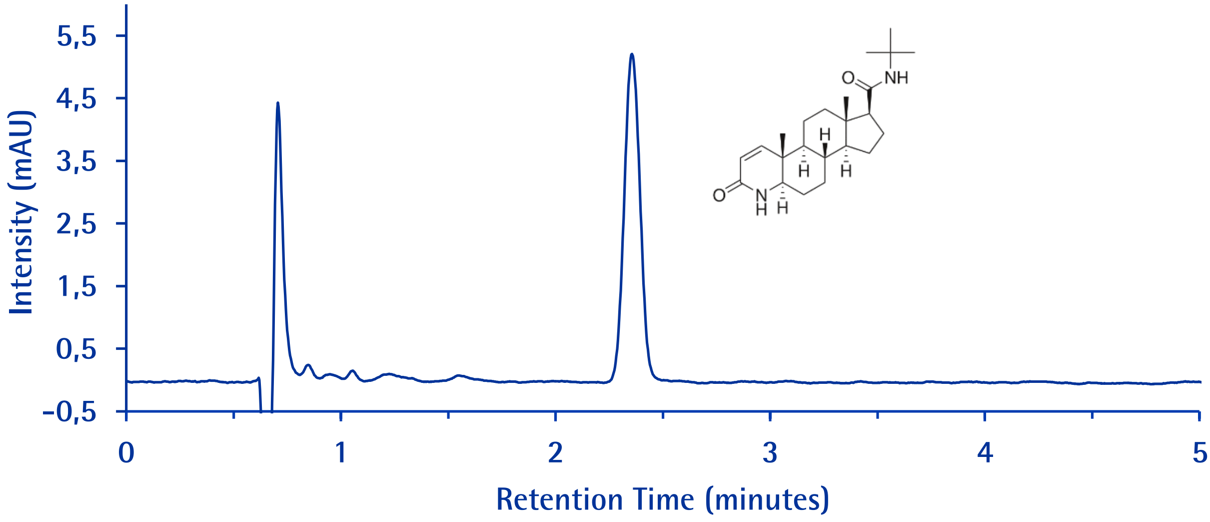
No	Compound	Retention Time (min)	Capacity factor	Plates	T_{USP}
1	T_0 (void volume)	1.1	-	-	-
2	Finasteride	3.5	2.3	6086	1.2

Finasteride Tablets –modified 2 (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV, 240 nm	
Cell:	11 µL	
Flow Rate:	2.25 mL/min	
Mobile Phase:	Acetonitrile and 2.5 mM phosphoric acid in Water 40:60 (v/v) (monograph text says 1:1)	
Temperature:	45 °C	
Diluent:	Acetonitrile and water 70:30 (v/v)	
Sample Solution:	Finasteride RS 100 µg/mL (100 ppm)	
Sample prep.:	Dissolve an accurately weighed quantity of USP Finasteride RS in Diluting solution, and dilute quantitatively, and stepwise if necessary, with Diluting solution to obtain a solution having a known concentration of about 100 µg per mL.	
Pressure Drop:	110 Bar (1595 psi)	



Chromatographic Data

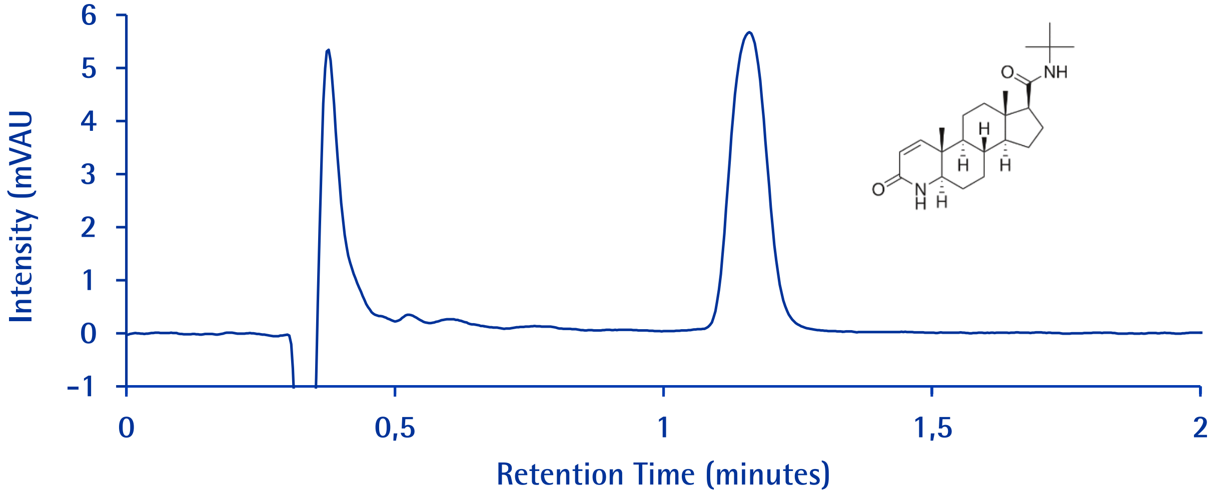
No	Compound	Retention Time (min)	Capacity factor	Plates	T_{USP}
1	T_0 (void volume)	0.7	-	-	-
2	Finasteride	2.4	2.3	4816	1.0

Finasteride Tablets –modified 3 (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 50x4.6 mm	1.52021.0001
Injection:	10 µL	
Detection:	UV, 240 nm	
Cell:	11 µL	
Flow Rate:	2.25 mL/min	
Mobile Phase:	Acetonitrile and 2.5 mM phosphoric acid in Water 40:60 (v/v) (monograph text says 1:1)	
Temperature:	45 °C	
Diluent:	Acetonitrile and water 70:30 (v/v)	
Sample Solution:	Finasteride RS 100 µg/mL (100 ppm)	
Sample prep.:	Dissolve an accurately weighed quantity of USP Finasteride RS in Diluting solution, and dilute quantitatively, and stepwise if necessary, with Diluting solution to obtain a solution having a known concentration of about 100 µg per mL.	
Pressure Drop:	80 Bar (1160 psi)	



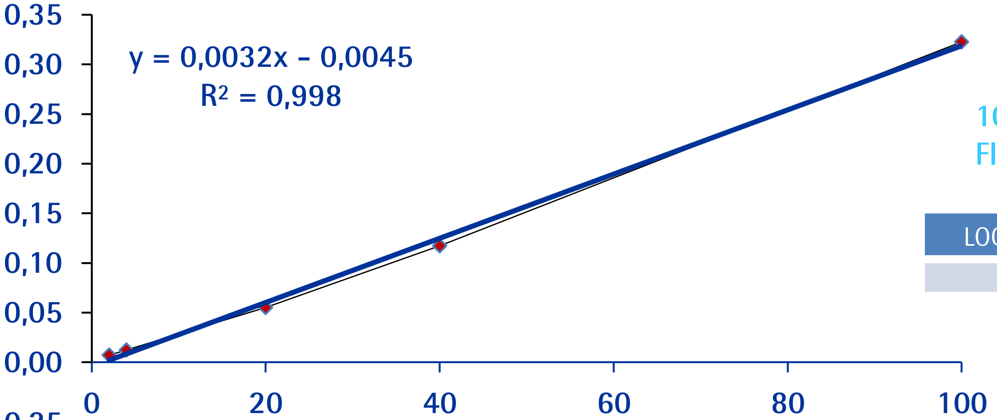
Chromatographic Data

No	Compound	Retention Time (min)	Capacity factor	Plates	T_{USP}
1	T_0 (void volume)	0.4	-	-	-
2	Finasteride	1.2	2.2	1543	1.1

Finasteride Tablets – modified (USP38-NF33)

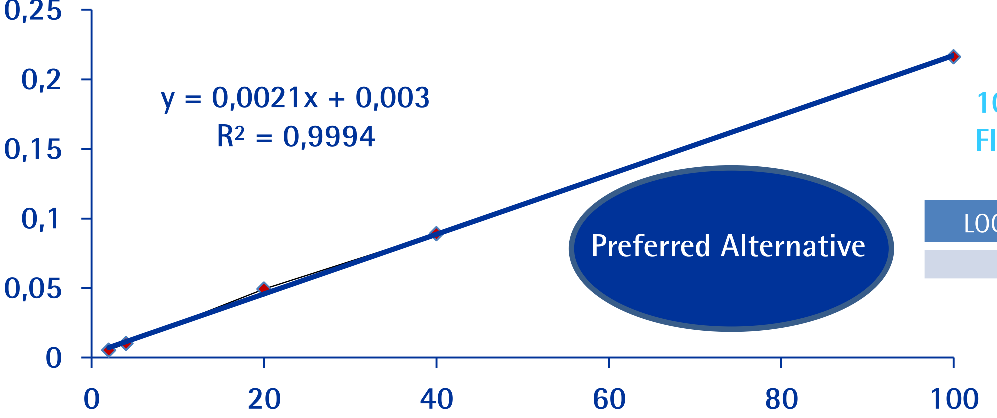
Chromolith® HighResolution RP-18 endcapped

Linearity: Analysis of repeated injections (n=5) of standard solutions of finasteride in the calibration range 2-100 ppm (µg/mL).



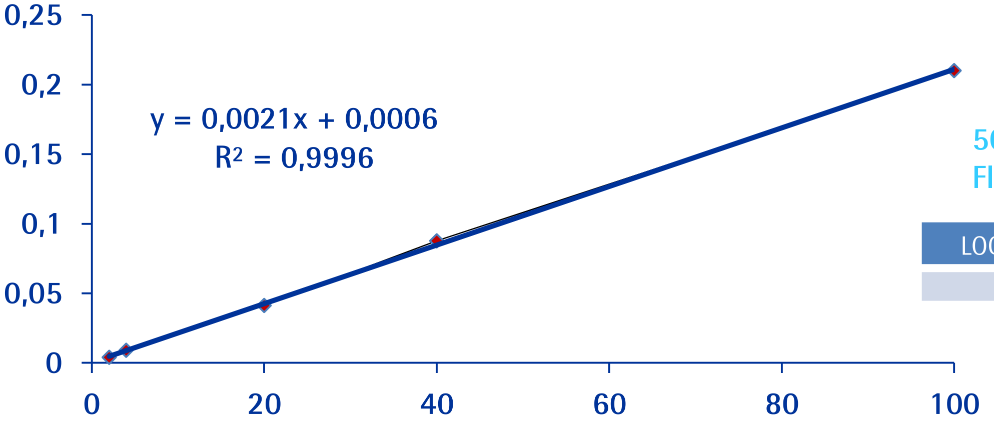
100 mm long column
Flow rate: 1.5 mL/min

LOQ (ppm)	LOD (ppm)
6.9	21.0



100 mm long column
Flow rate: 2.25 mL/min

LOQ (ppm)	LOD (ppm)
3.8	11.5

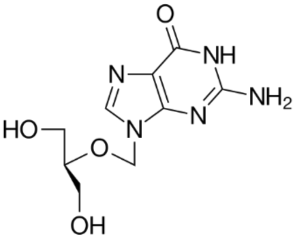


50 mm long column
Flow rate: 2.25 mL/min

LOQ (ppm)	LOD (ppm)
3.1	9.5

Ganciclovir for Injection (USP38-NF33)

The following pages is an example of a monograph that is differing from the active pharmaceutical monograph and where we have used a monolithic column with same geometry and modification as described in the official text.



The monograph specify a 100x4.6 mm column with L1 (RP-18) packing operating at 1.2 mL/min. In this monograph no particle size is referred, hence we focus on the suitability criteria only;

- **The relative retention times for hypoxanthine and ganciclovir are about 0.7 and 1.0**
- **Resolution: NLT 3.0 between hypoxanthine and ganciclovir**
- **Column efficiency: NLT 1000 theoretical plates**
- **Tailing factor: NMT 2.0**

An alternative method was developed, with faster flow rate.

Ganciclovir for Injection (USP38-NF33)

(USP38–NF33 Page 3653 - Pharmacopeial Forum: Volume No. 29(3) Page 630

Mobile phase: 1.4 g/L of monobasic ammonium phosphate & 2.0 g/L of phosphoric acid in water

Internal standard solution: 0.15 mg/mL of hypoxanthine in water

Standard stock solution: 250 µg/mL of USP Ganciclovir RS in water

Standard solution: 0.05 mg/mL of USP Ganciclovir RS prepared as follows. Transfer 20.0 mL of the Standard stock solution and 10.0 mL of the Internal standard solution to a 100-mL volumetric flask. Dilute with Mobile phase to volume.

Sample stock solution: Nominally 1 mg/mL of ganciclovir, from Ganciclovir for Injection in water

Sample solution: Nominally 0.05 mg/mL of ganciclovir prepared as follows. Transfer 5.0 mL of Sample stock solution and 10.0 mL of Internal standard solution to a 100-mL volumetric flask, and dilute with Mobile phase to volume.

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 254 nm
Column: 100x4.6 mm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL

System suitability (Sample: Standard solution)

The relative retention times for hypoxanthine and ganciclovir are about 0.7 and 1.0, respectively.

Suitability requirements

1. Resolution: NLT 3.0 between hypoxanthine and ganciclovir

2. Column efficiency: NLT 1000 theoretical plates

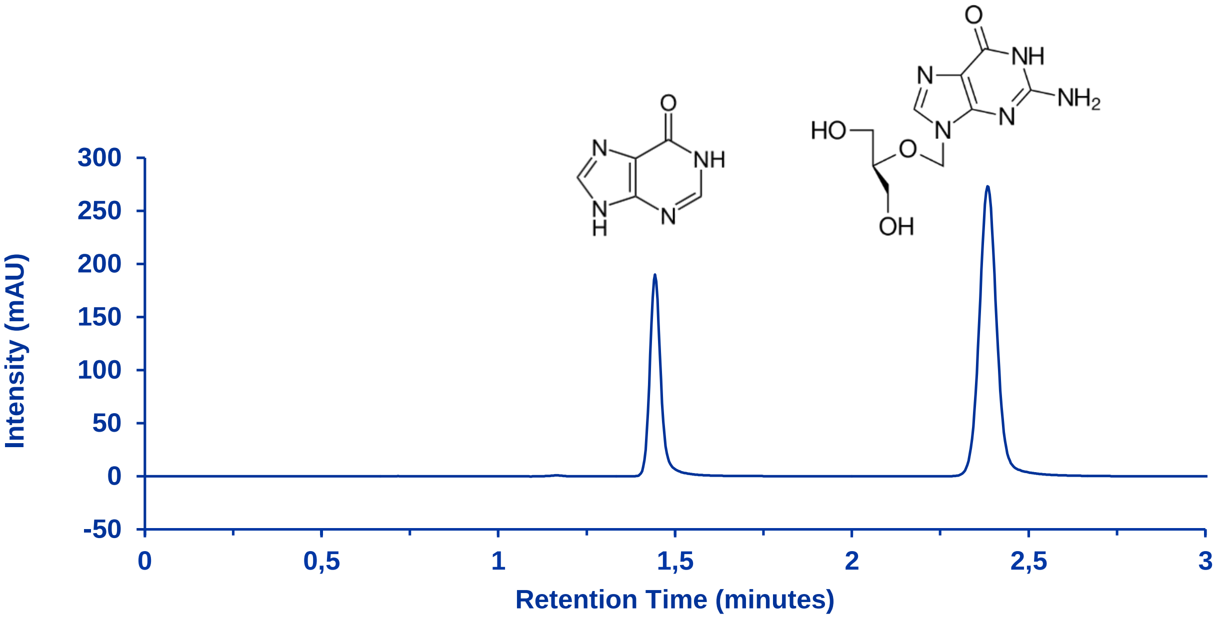
3. Tailing factor: NMT 2.0

Ganciclovir Injection Solution (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV, 254 nm	
Cell:	11 µL	
Flow Rate:	2.0 mL/min	
Mobile Phase:	1.4 g/L of monobasic ammonium phosphate and 2.0 g/L of o-phosphoric acid (85%) in water	
Temperature:	Ambient	
Diluent:	Water	
Sample:	Ganciclovir RS, 0.05 mg/mL of USP Ganciclovir RS prepared as follows. Transfer 20.0 mL of standard stock solution (250 µg/mL of USP Ganciclovir RS in water) and 10.0 mL of the Internal standard solution to a 100 mL volumetric flask. Dilute with mobile phase to volume	
Pressure Drop:	97 bar (1407 psi)	



Chromatographic Data

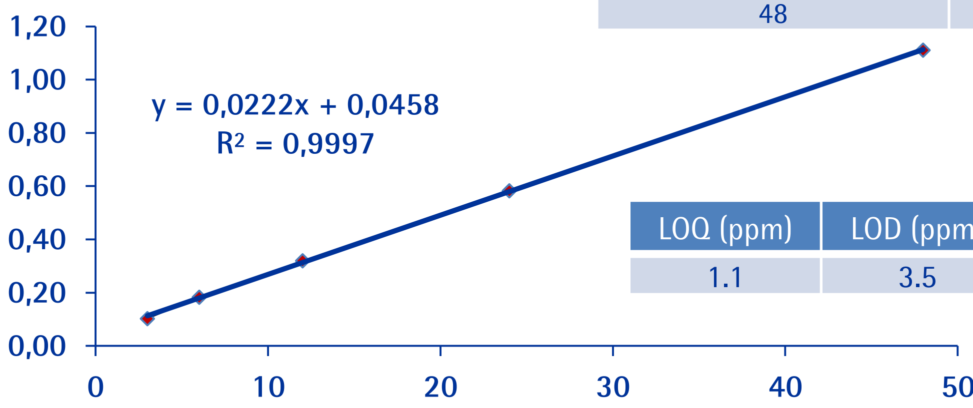
No	Compound	Retention Time (min)	RRT	Plates	T_{USP}	Resolution
1	T_0 (void volume)	0.7	-	-	-	-
2	Hypoxanthine	1.4	0.6	11153	1.2	-
3	Ganciclovir RS	2.4	1.0	11560	1.1	13

Ganciclovir Injection Solution (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Linearity – Hypoxanthine: Analysis of repeated injections (n=5) of standard solutions in the calibration range 3-48 ppm (µg/mL).

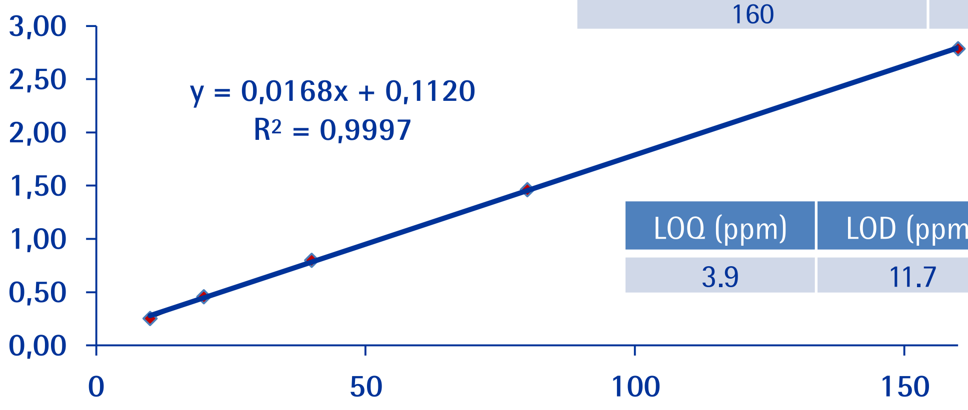
Concentration (ppm)	Area
3	0,10
6	0,18
12	0,32
24	0,58
48	1,11



LOQ (ppm)	LOD (ppm)
1.1	3.5

Linearity – Ganciclovir: Analysis of repeated injections (n=5) of standard solutions in the calibration range 10-160 ppm (µg/mL).

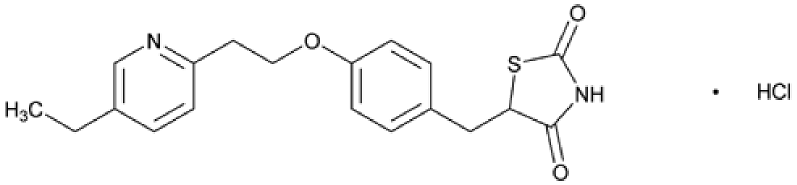
Concentration (ppm)	Area
10	0,25
20	0,46
40	0,80
80	1,46
160	2,79



LOQ (ppm)	LOD (ppm)
3.9	11.7

Pioglitazone Hydrochloride (USP38-NF33)

The following pages is another example where the USP monograph is first developed with a particle packed column fitting the monograph descripition and thereafter transferred to the same geometry (150x4.6 mm) monolithic column.



The monograph specify a 150x4.6 mm column with L1 (RP-18) packing having 5 µm particles operating at 1.5 mL/min. When the particle size and/or column backbone is changed, the flow rate may require adjustment, for the same performance (as measured by reduced plate height). Flow rate changes can be made by using the equation on page 15 or it can be adjusted by ±50%. Here, the flow rate is maintained as in the monograph and you can witness that the resolution is better and run time is shorter with the monolithic column. All performance criteria is fulfilled with both columns.

The following benefits can be attained in transferring from particle to monolithic column:

a) The method runs faster with higher resolution.

b) Lower backpressure on the monolithic column = less stress on HPLC system

Pioglitazone Hydrochloride (USP38-NF33)

Mobile phase

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

Standard solution

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

System suitability stock solution

0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol

System suitability solution

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

Sample solution

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

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

Detector: UV 269 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 25 ± 2.5°C

Flow rate: 0.7 mL/min

Injection size: 20 µL

[Note—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

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

- Approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.

Suitability requirements

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

Analysis (Samples: Standard solution and Sample solution)

Calculate the percentage of $C_{19}H_{20}N_2O_3S·HCl$ in the portion of Pioglitazone Hydrochloride taken:

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

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

Acceptance criteria:

98.0%–102.0% on the dried basis

Pioglitazone Hydrochloride (USP38-NF33)

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

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

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

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

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

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

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

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

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

Pioglitazone Hydrochloride (USP38-NF33)

Acceptance criteria

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

Table 1.

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

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

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

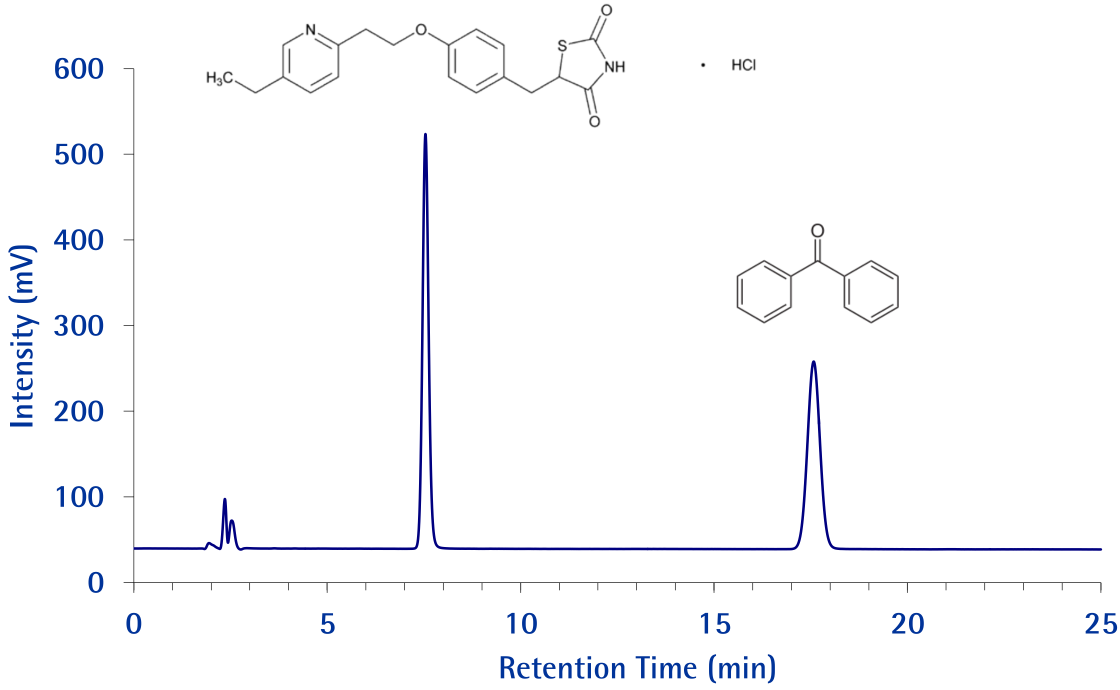
c = (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione.

Pioglitazone Hydrochloride (USP38-NF33)

Particle packed RP-18 endcapped column

Chromatographic Conditions

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



Chromatographic Data

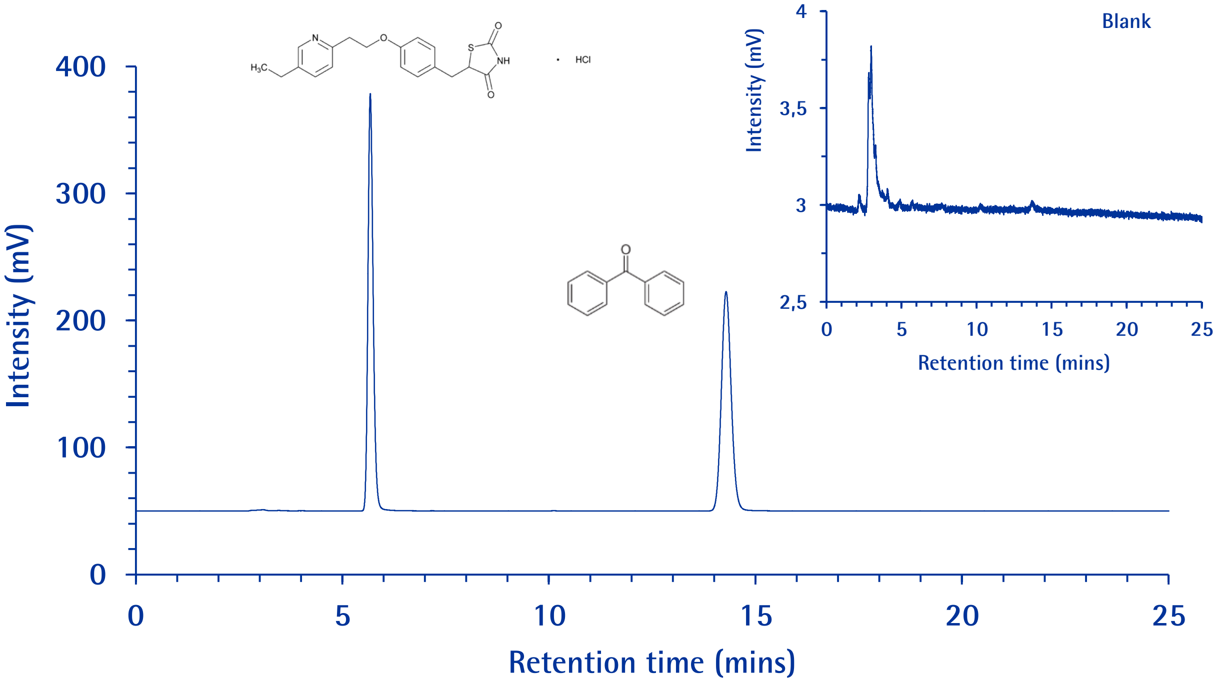
No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Pioglitazone HCl	7.6	1.0	0.0	1.09
2	Benzophenone (SST)	17.4	2.3	22.0	1.03

Pioglitazone Hydrochloride (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	10 µL	
Detection:	UV, 269 nm	
Cell:	10 µL	
Flow Rate:	0.7 mL/min	
Mobile Phase:	Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid, 25:25:1 (v/v/v)	
Temperature:	25 °C	
SST stock:	0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol	
SST solution:	Dilute the System suitability stock solution with Mobile phase to obtain a solution containing 25 µg/mL of pioglitazone hydrochloride &	
Pressure Drop:	49 Bar (710 psi)	

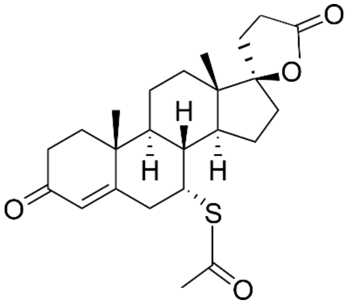


Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Tailing factor	Resolution
1	Pioglitazone	5.7	-	1.2	0
2	Benzophenone	14.3	2.5	1.1	25.9

Spironolactone (USP38-NF33)

The following pages is an example where the USP monograph has a very limited system suitability criteria and where you potentially can make big changes without a complete revalidation.



The monograph (assay only) specify a 150x4.6 mm column with L1 (RP-18) packing operating at 1.0 mL/min. The only system suitability criteria is the tailing factor; should not be more than 2.0 On the following pages you can find examples first with 150, 100 and 50 mm long columns at 1.0 and 1.5 mL/min flow rate.

So which example should be chosen?

The 150x4.6 at 1.0 mL/min follow the monograph exactly, and the 50 mm long column with an elevated flow is the quickest...but it is really up to you to choose which one fits your purpose....

All examples provide a peak shape much better than 2.0, hence passes the criteria.

Spironolactone (USP38-NF33)

(USP38–NF33 Page 5348 - Pharmacopeial Forum: Volume No. 37(1)

ASSAY – Procedure

Mobile phase: Methanol and water (60:40)

Standard solution: 0.5 mg/mL of Spironolactone RS in a mixture of acetonitrile and water (1:1)

Sample solution: 0.5 mg/mL of Spironolactone in a mixture of acetonitrile and water (1:1)

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 230 nm
Column: 150x4.6 mm; packing L1
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability (Sample: Standard solution)
Suitability requirements
Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.5%

Analysis
Samples: Standard solution and Sample solution

Calculate the percentage of spironolactone ($C24H32O4S$) in the portion of sample 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 Spironolactone RS in the Standard solution (mg/mL)
CU = = concentration of Spironolactone in the Sample solution (mg/mL)

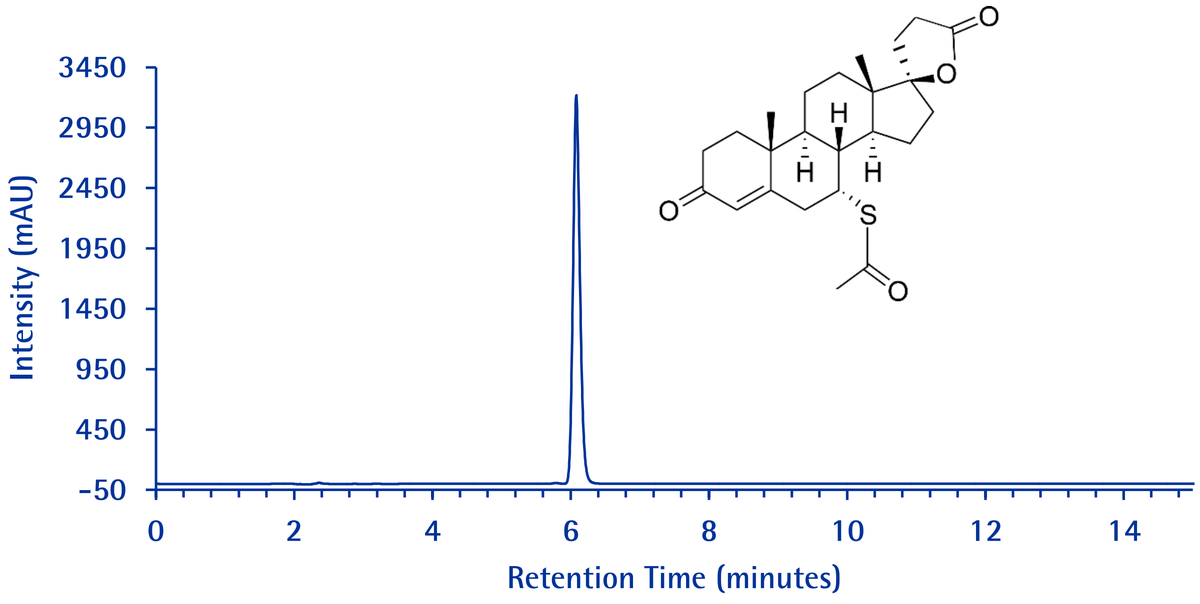
Acceptance criteria: 97.0%–103.0% on the dried basis

Spironolactone (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	20 µL	
Detection:	UV, 230 nm	
Cell:	11 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Water and methanol 40:60 (v/v)	
Temperature:	Ambient	
DiluentL:	Acetonitrile/Water 1:1 (v/v)	
Sample Solution:	0.5 mg/mL (500 ppm) of Spironolactone RS in diluent	
Pressure Drop:	67 bar (972 psi)	



Chromatographic Data

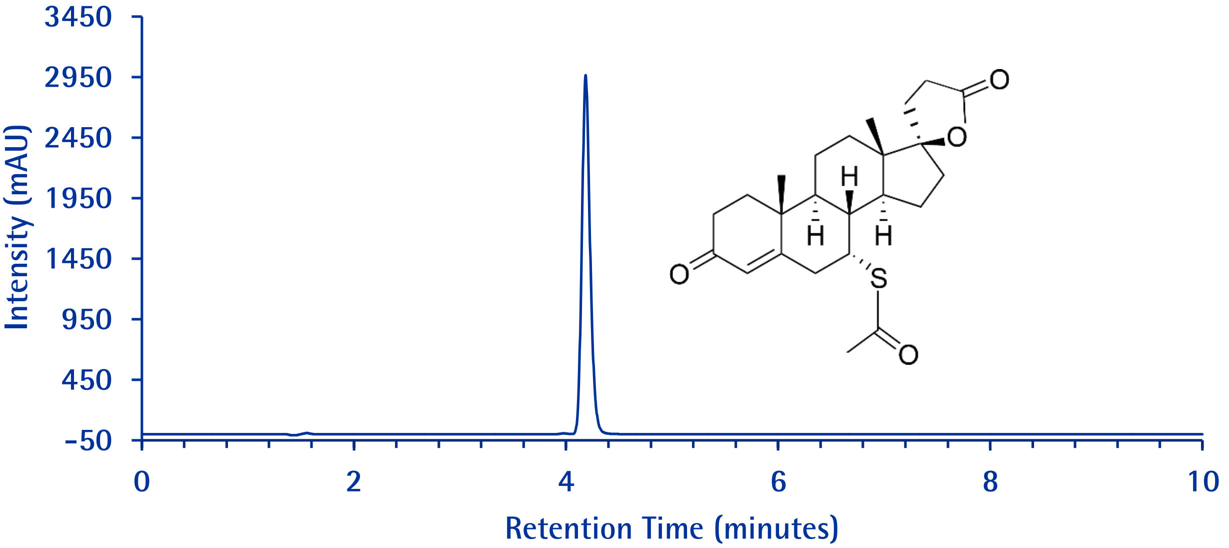
No.	Compound	Retention Time (min)	Capacity factor (k)	Plates	T_{USP}
1	t'o (void volume)	1.9	0	-	-
2	Spironolactone	6.1	2.3	17219	1.1

Spironolactone – modified 1 (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	20 µL	
Detection:	UV, 230 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Water and methanol 40:60 (v/v)	
Temperature:	Ambient	
DiluentL:	Acetonitrile/Water 1:1 (v/v)	
Sample Solution:	0.5 mg/mL (500 ppm) of Spironolactone RS in diluent	
Pressure Drop:	110 bar (1595 psi)	



Chromatographic Data

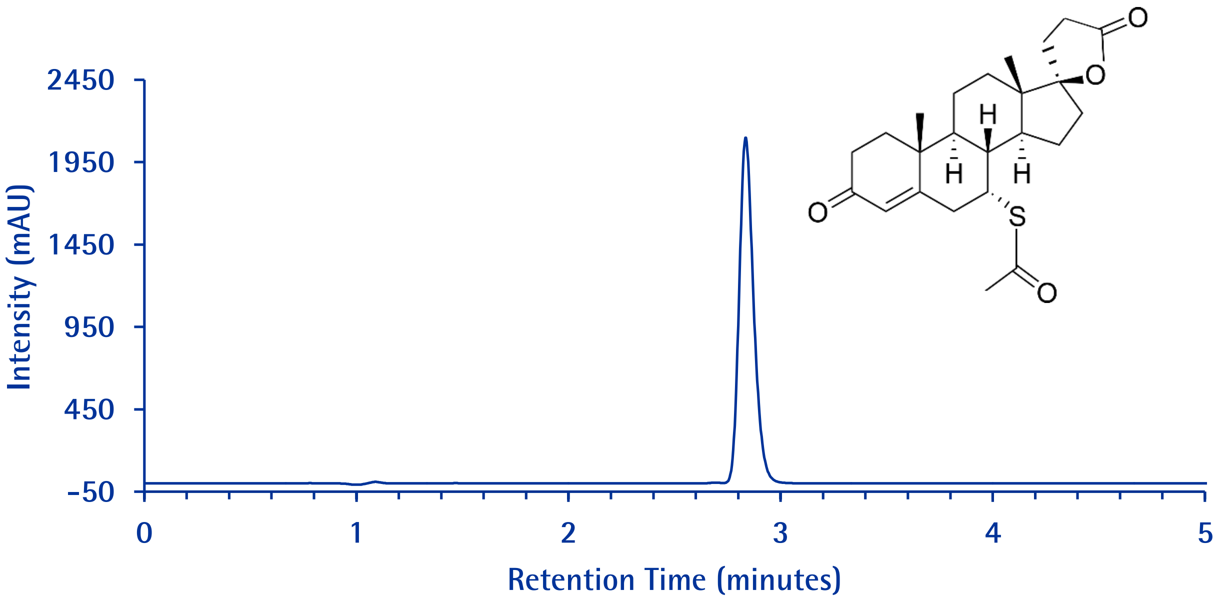
No.	Compound	Retention Time (min)	Capacity factor (k)	Plates	T_{USP}
1	t'o (void volume)	1.4	0	-	-
2	Spironolactone	4.2	2.1	15938	1.2

Spironolactone – modified 2 (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022.0001
Injection:	13 µL (reduced with 33% as it is scaled to a 33% shorter column with same diameter)	
Detection:	UV, 230 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Water and methanol 40:60 (v/v)	
Temperature:	Ambient	
DiluentL:	Acetonitrile/Water 1:1 (v/v)	
Sample Solution:	0.5 mg/mL (500 ppm) of Spironolactone RS in diluent	
Pressure Drop:	81 bar (1175 psi)	



Chromatographic Data

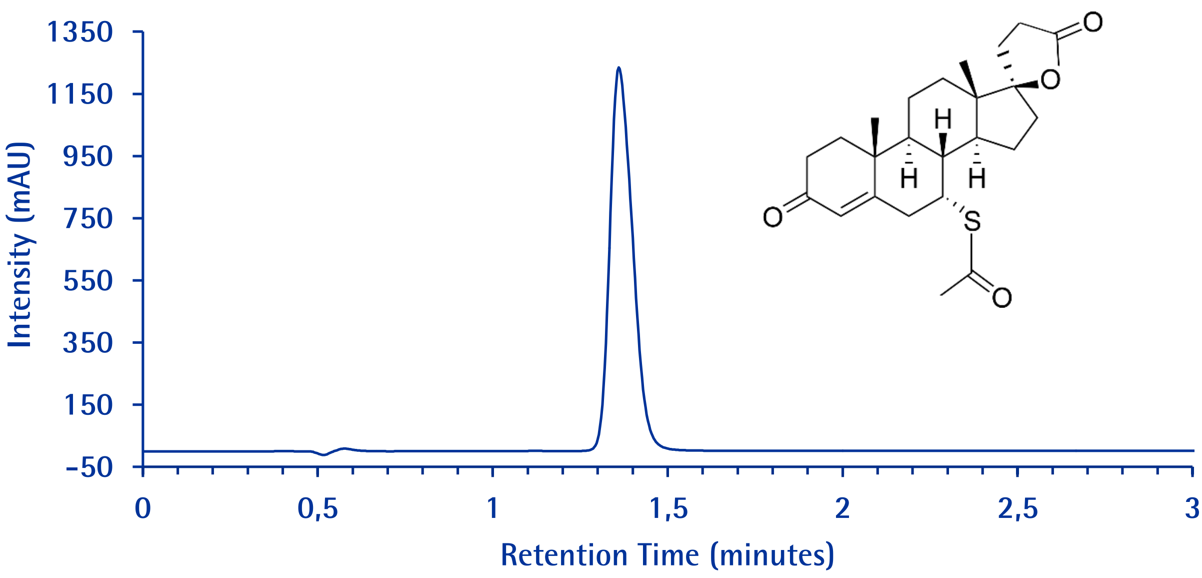
No.	Compound	Retention Time (min)	Capacity factor (k)	Plates	T_{USP}
1	t'o (void volume)	0.9	0	-	-
2	Spironolactone	2.8	2.1	9936	1.0

Spironolactone – modified 3 (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 50x4.6 mm	1.52021.0001
Injection:	7 µL (reduced with 67% as it is scaled to a 67% shorter column with same diameter)	
Detection:	UV, 230 nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Water and methanol 40:60 (v/v)	
Temperature:	Ambient	
DiluentL:	Acetonitrile/Water 1:1 (v/v)	
Sample Solution:	0.5 mg/mL (500 ppm) of Spironolactone RS in diluent	
Pressure Drop:	59 bar (856 psi)	



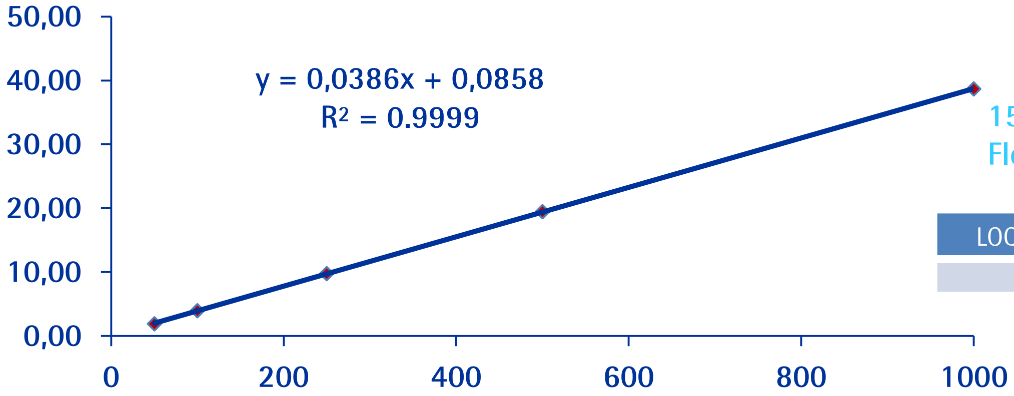
Chromatographic Data

No.	Compound	Retention Time (min)	Capacity factor (k)	Plates	T_{USP}
1	t'o (void volume)	0.4	0	-	-
2	Spironolactone	1.4	2.3	2010	1.3

Spironolactone (USP38-NF33)

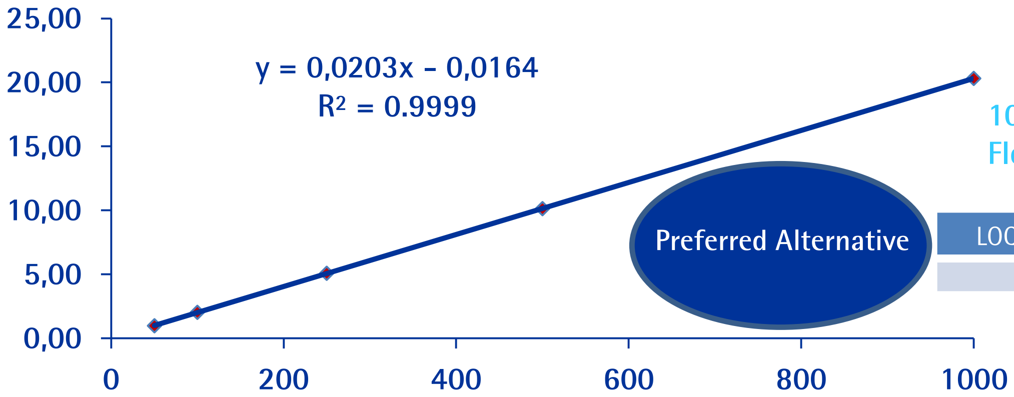
Chromolith® HighResolution RP-18 endcapped

Linearity: Analysis of repeated injections (n=5) of standard solutions of finasteride in the calibration range 2-100 ppm (µg/mL).



150 mm long column
Flow rate: 1.0 mL/min

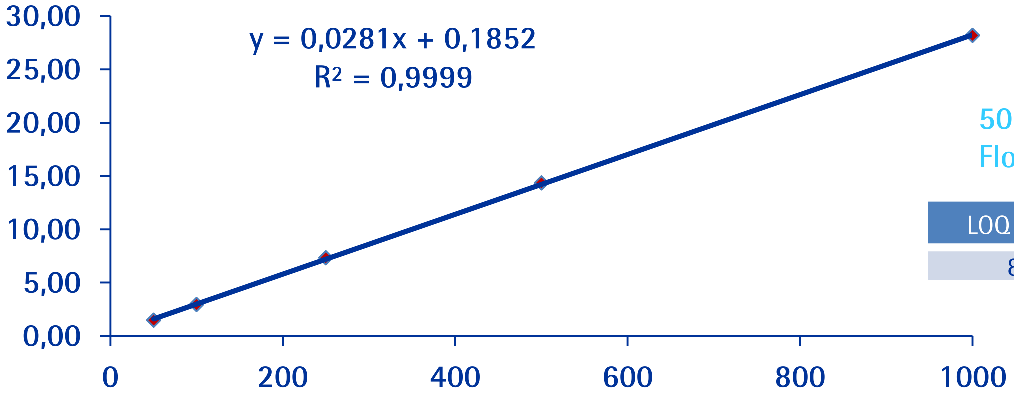
LOQ (ppm)	LOD (ppm)
4.3	14



Preferred Alternative

100 mm long column
Flow rate: 1.5 mL/min

LOQ (ppm)	LOD (ppm)
3.1	9.3

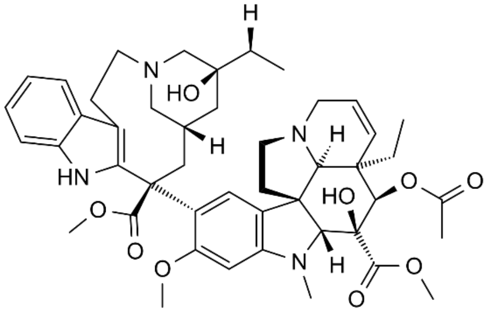


50 mm long column
Flow rate: 1.5 mL/min

LOQ (ppm)	LOD (ppm)
8.5	26

Vinblastine Sulfate (USP38-NF33)

The following pages is an example where the USP monograph has a very limited system suitability criteria and where you potentially can make big changes without a complete revalidation.



The monograph (same for both assay and impurities) specify a 150x4.6 mm column with L1 (RP-18) packing operating at 2 mL/min. The only system suitability criteria is that the resolution should be not less than (NLT) 4.0 between vincristine and vinblastine using the system suitability solution. On the following pages you can find a method with a 150x4.6 mm monolithic column that is compliant with set requirements.

This monograph was not possible to scale to shorter column dimensions due to all minor impurities in the samples that would co-elute on columns with less separation efficiency, and loss of sufficient resolution between vincristine and vinblastine .

Vinblastine Sulfate (USP38-NF33)

(USP38–NF33 Page 5780 - Pharmacopeial Forum: Volume No. 32(5) Page 1470

ASSAY – Procedure

Solution A: Diethylamine and water (14:986). Adjust with phosphoric acid to a pH of 7.5.
Solution B: Acetonitrile and methanol (20:80)
Mobile phase: Solution A and Solution B (38:62)

Standard solution: 0.4 mg/mL of USP Vinblastine Sulfate RS in water

System suitability solution: 0.4 mg/mL each of vincristine sulfate and vinblastine sulfate in water prepared as follows. Transfer USP Vincristine Sulfate RS or USP Vincristine Sulfate (Assay) RS to a suitable volumetric flask, and dissolve in the Standard solution.

Sample solution: 0.4 mg/mL of Vinblastine Sulfate in water

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 262 nm
Precolumn: Packed with porous silica gel; installed between the pump and the injector
Analytical: 150x4.6 mm; packing L1
Flow rate: 2 mL/min
Injection size: 20 µL

System suitability (Samples: Standard solution and System suitability solution)

Suitability requirements
Resolution: NLT 4.0 between vincristine and vinblastine, System suitability solution
Relative standard deviation: NMT 2.0%, Standard solution

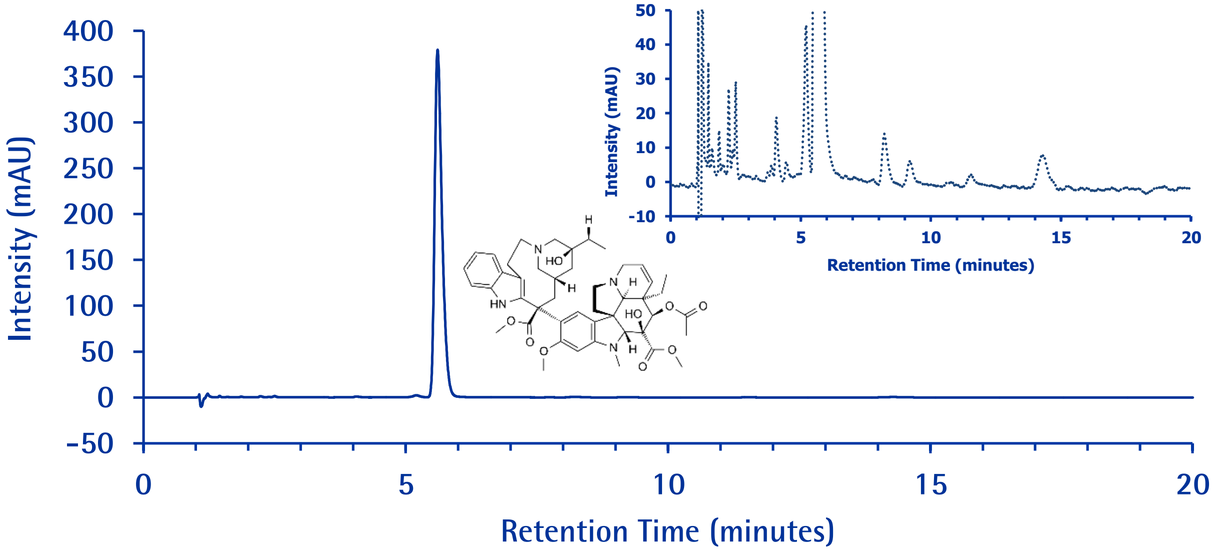
Organic Impurities
Mobile phase, Standard solution, System suitability solution, and System suitability: Proceed as directed in the Assay.
Sample solution A: Use the Sample solution prepared in the Assay.
Sample solution B: 16 µg/mL of vinblastine sulfate in water from Sample solution A
Only difference - Injection size: 200 µL (20 µL for System suitability)

Vinblastine Sulfate (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	20 µL	
Detection:	Dionex Ultimate 3000 VWD-3400, 5 Hz, Response Time 0.1 s, UV = 262 nm	
Cell:	11 µL	
Flow Rate:	2 mL/min	
Mobile Phase A:	Dimethylamine and water 14/986 (v/v). Adjusted with phosphoric acid to a pH of 7.5	
Mobile Phase B:	Acetonitrile and Methanol 20/80 (v/v)	
Isocratic mixture:	Mobile phase A and B 38:62 (v/v)	
Temperature:	Ambient	
Diluent:	Water	
Sample Solution:	0.4 mg/mL of USP Vinblastine Sulfate RS in water	
Pressure Drop:	140 bar (2030 psi)	



Chromatographic Data

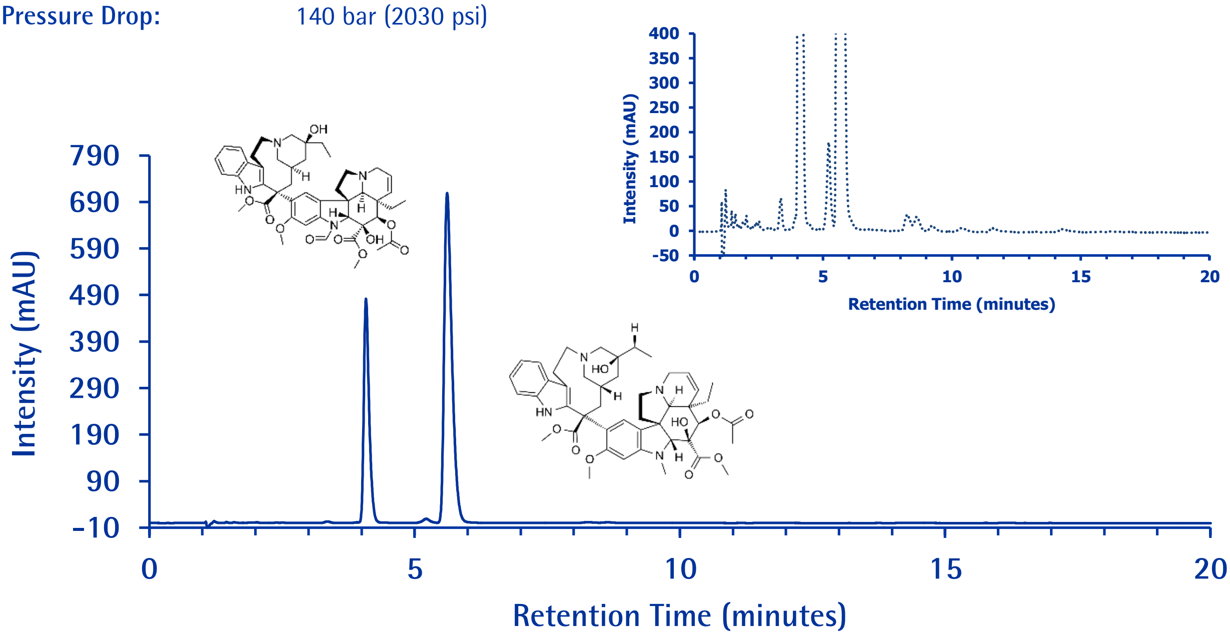
No.	Compound	Retention Time (min)	Resolution	Plates	T_{USP}
1	t'o (void volume)	1.2	-	-	-
2	Vinblastine Sulfate	5.6	-	8395	1.4

Vinblastine Sulfate (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped 150x4.6 mm	1.52023.0001
Injection:	20 µL	
Detection:	UV, 262 nm	
Cell:	11 µL	
Flow Rate:	2 mL/min	
Mobile Phase A:	Dimethylamine and water 14/986 (v/v). Adjusted with phosphoric acid to a pH of 7.5	
Mobile Phase B:	Acetonitrile and Methanol 20/80 (v/v)	
Isocratic:	A / B 38/62 (v/v)	
Temperature:	Ambient	
Diluent:	Water	
Sample:	0.4 mg/mL each of Vincristine sulfate and Vinblastine Sulfate in water prepared as follows. Transfer USP Vincristine Sulfate RS (or Assay RS) to a suitable volumetric flask, and dissolve in the Standard solution.	
Pressure Drop:	140 bar (2030 psi)	



Chromatographic Data

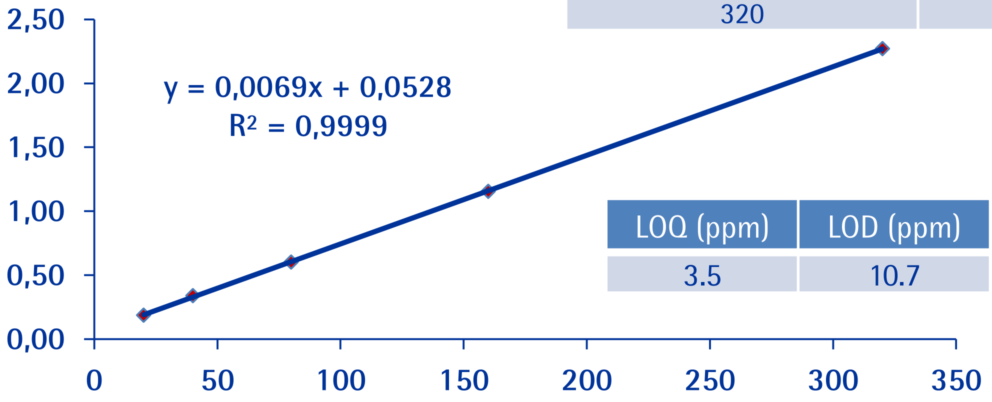
No.	Compound	Retention Time (min)	Resolution	Plates	T_{USP}
1	t'o (void volume)	1.2	-	-	-
2	Vincristine Sulfate	4.1	-	12102	1.4
3	Vinblastine Sulfate	5.6	8.0	8498	1.4

Vinblastine Sulfate (USP38-NF33)

Chromolith® HighResolution RP-18 endcapped

Linearity – Vincristine: Analysis of repeated injections (n=5) of standard solutions in the calibration range 20-320 ppm (µg/mL).

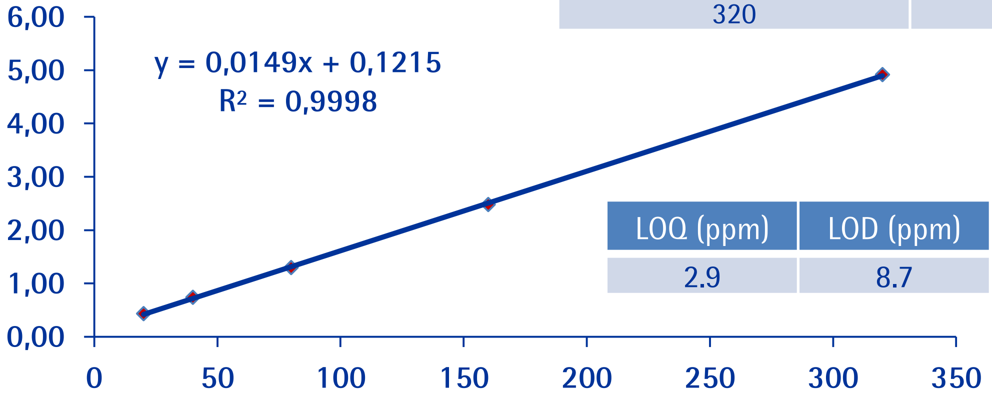
Concentration (ppm)	Area
20	0,19
40	0,34
80	0,60
160	1,16
320	2,27



LOQ (ppm)	LOD (ppm)
3.5	10.7

Linearity – Vinblastine: Analysis of repeated injections (n=5) of standard solutions in the calibration range 20-320 ppm (µg/mL).

Concentration (ppm)	Area
20	0,43
40	0,74
80	1,30
160	2,48
320	4,91



LOQ (ppm)	LOD (ppm)
2.9	8.7