Pharmacopoeia
Monograph Methods

HPLC and UHPLC methods for Regulated Drug Analysis

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Content

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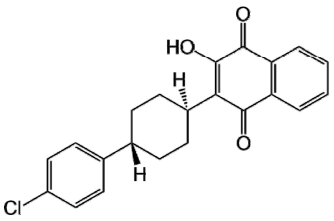

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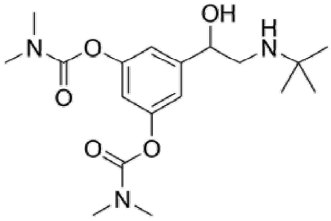

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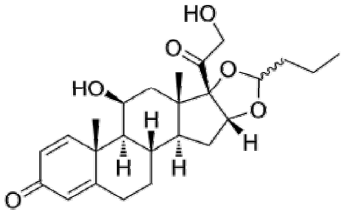

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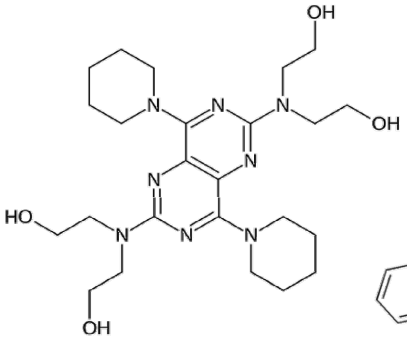

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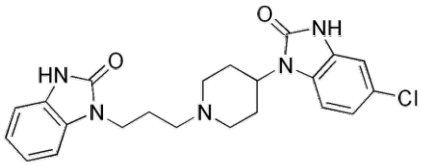

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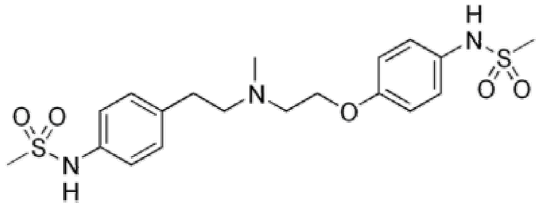

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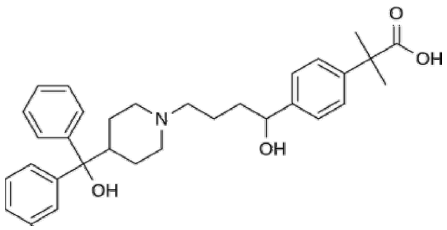

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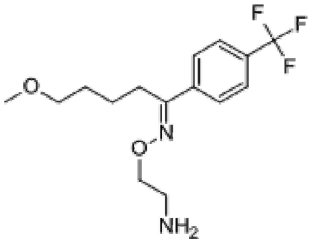

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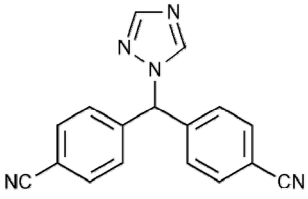

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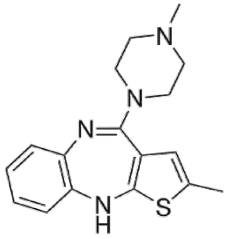

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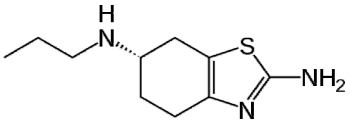

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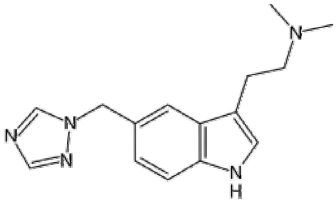


Rizatriptan

Application Index

Introduction

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.

Generic drugs of biological type are commonly known as biosimilars, and they are different from chemical drugs because of its biological nature and thus regulated under extended set of rules. Biosimilars, or follow-on biologics, are biologic medical products whose active drug substance is made by a living organism. Due to the higher complexity with biological drug manufacturing; small differences in impurities and/or breakdown products can have serious health implications. Thus only few biosimilars have been approved to this date. However, biosimilars is a rather "hot topic" and something currently pursued by numerous companies around the world. In most cases, generic products are available as soon as the patent protection afforded to the original developer have expired. The added market competition with approved generics often lead to substantially lower prices for both the ethical drug product and the generic forms.

In this compilation we have developed a number of HPLC methods for generic small molecule drugs following current monograph methods in the European Pharmacopoeia (EP) and the United States Pharmacopoeia (USP). In particular this compilation presents new data on peak purity analysis, as well as an extended discussion about the differences among partial and full validation of monograph methods depending if the new method are within allowed changes per the pharmacopoeia. A complete example is shown for method validation within allowed changes (Letrozole and Related Substances), as well as allowed scaling of methods from particulate to monolithic columns. There are also examples of NOT allowed method changes, from HPLC to UHPLC conditions (gradient methods), with substantial time saving in all examples. The benefits with the new methods are obvious and should stimulate you to consider such method transfer despite it would require a complete method revalidation.

In all examples, we have used high quality HPLC and UHPLC columns from Merck Millipore combined with our high purity solvents and reagents. Thus examples are given to where the Purospher® STAR (RP-18 endcapped, RP-8 endcapped, and Phenyl stationary phases) as well as Chromolith® HighResolution RP-18 endcapped columns can easily be introduced and used for your needs in validated pharmaceutical quality control.

Pharmacopoeial Requirements

The regulation of medicinal products date back several hundred years. In the late 15th century King Henry VIII gave the Royal College of Physicians of London the power to inspect apothecaries' products in the London area and to destroy defective stock. The first list of approved drugs (with manufacturing guidelines) was published in 1618 by the London Pharmacopoeia, and the first edition of the British Pharmacopoeia (BP) was published in 1864 being one of the first attempts to harmonize pharmaceutical standards, through the merger of the London, Edinburgh and Dublin Pharmacopoeias. Today, we can purchase certified reference standards from several official bodies.

The United States Pharmacopoeia (USP) was founded in 1820. Over the last two centuries massive progress has been made around the globe to make drugs safe for consumers, both on a national as well as on an international level, thus assuring the potency of commercially available drug products. Depending where (in which country or region) a finalized drug product will be used it must meet the specified regulations and thus many pharmaceutical companies work from several pharmacopoeias. In this chapter the European (EP) and United States Pharmacopoeia (USP) will be discussed.

One of the more important improvements is the requirement for impurity profiling, i.e. the analysis of related substances (RS) monographs. At present, various regulatory authorities like the International Conference of Harmonization (ICH), European Directorate for the Quality of Medicines and Healthcare (EDQM), United States Food and Drug Administration (USFDA), and the Canadian Drug and Health Agency are all emphasizing the purity requirements and the identification of impurities in Active Pharmaceutical Ingredients (APIs), and formulated drugs. The ICH Harmonized Tripartite Guideline, 'Impurities in new drug substances', Q3A(R2), states that all actual and likely impurities in a new drug substance should be summarized and laboratory studies conducted to detect these impurities. 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. That said, assay and potency methods of pharmaceuticals are likely the most common chromatographic analysis around the world. The requirements for an assay are less stringent than for an impurity profiling. Contaminants need only be separated from the main component and the focus is more on accuracy, precision, range and linearity.

Figure 1. The Logotypes of British (BP), European (EP), Indian (IPC), and United States (USP) Pharmacopoeias.

Assay (potency) methods as well as the related substances monographs (identification of impurities) are both carried out with various analytical techniques, and where different chromatographic and spectroscopic techniques are common; either alone or in combination with other techniques. Thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), gas chromatography (GC), high performance liquid chromatography (HPLC), and atomic absorption spectroscopy (AAS) in addition to more classical tests based on titration are commonly used. HPLC has especially been widely exploited for impurity profiling methods. The reasons for this are the wide range of detectors available that connect easily with HPLC along with the variety of column chemistries (stationary phases) commercially available. Very simply, with HPLC it is possible to develop robust and reliable methods having necessary sensitivity and linearity that meet requirements in selectivity and provide cost effectiveness to the laboratory.

On the following pages, information from USP and EP will be used and explained. A monograph method represents published standards by which the use of one or more substances is automatically authorized. Thus by following the specific method and complying with set specifications a manufacturer can prove the safety of their products. Thus, examples are shown herein how validation of monograph methods can be carried out, and remember, Merck Millipore analytical chromatography columns can be an excellent choice for your assay and/or impurity-profiling needs.

Changing a Regulated Method

What changes are allowed in a monograph method?

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

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

Factors that may affect chromatographic behavior:

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

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

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

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

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

	USP	EP
Column Length**	See separate instructions on next page. NEW in USP 37!	± 70%
Column Inner Diameter	See separate instructions on next page. NEW in USP 37!	± 25%
Particle Size	See separate instructions on next page. NEW in USP 37!	Reduction of 50%, no increase
Flow rate	See separate instructions on next page. NEW in USP 37!	± 50%
Column Temperature	± 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 for both isocratic and gradient separations	± 0.2 units
UV Wavelength	No adjustment is permitted	No adjustment is permitted
Buffer salts Concentration	± 10% if the permitted pH variation (see above) 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 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 (e.g., C18).
In any case, all system suitability requirements specified in the official procedure must be met with the guard column installed.

Changes in USP37

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

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

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

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

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

Changes in F, dc, and dp are not allowed for gradient separations.
Additionally, the flow rate can be adjusted by ±50% (isocratic only). EXAMPLES: Adjustments in column length, internal diameter, particle size, and flow rate can be used in combination to give equivalent conditions (same N), but with differences in pressure and run time. The following table lists some of the more popular column configurations to give equivalent efficiency (N), by adjusting these variables.

Changes in USP37

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

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

Injection Volume (HPLC):

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

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

Ratio of Components in Mobile Phase

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

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

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

Wavelength of UV-Visible Detector

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

Choosing the right Column to meet Monograph Specifications

The HPLC column choice is a very important consideration or it will be difficult to meet the set requirements in a monograph method. In the chapter discussing column selection, we have outlined which USP classification (code) our HPLC columns belong to.
At present, 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".*

USP Packings (L classifications)

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

A discussion with USP was initiated early in 2014, to further extend the inclusion of...or a monolithic rod to the L9, L10, L11 and L20 packings definition. This update is not yet in practice.

We at Merck Millipore have shown 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. Also it important to keep in mind that those columns mentioned in USP as monograph columns is not bound text – the actual monograph only describe the column geometry and classification.

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.

Atovaquone Oral Suspension (USP37–NF32) – Case Study

The current monograph for Atovaquone oral suspension is based on HPLC where the liquid chromatograph is equipped with a UV detector (220 nm) and a 125x4.6 mm column that contains packing L1 (RP-18/ODS) operated at 3.0 mL/min. No specific particle size is mentioned in the monograph.

At this flow rate, with normal particulate columns (5 µm), the back pressure over the HPLC system will be very high on a fresh column such that after a few runs, it will exceed the set backpressure limit and causes failure in analysis.

For these reasons we have transferred the current method to a 100x4.6 mm id monolithic column, Chromolith® HighResolution RP-18 endcapped column, see Figure 2, where the back pressure is only around 100 bar (1440 psi) under given experimental conditions. A pressure that any HPLC system easily can accommodate, and thereby provide robust and reliable results over time.

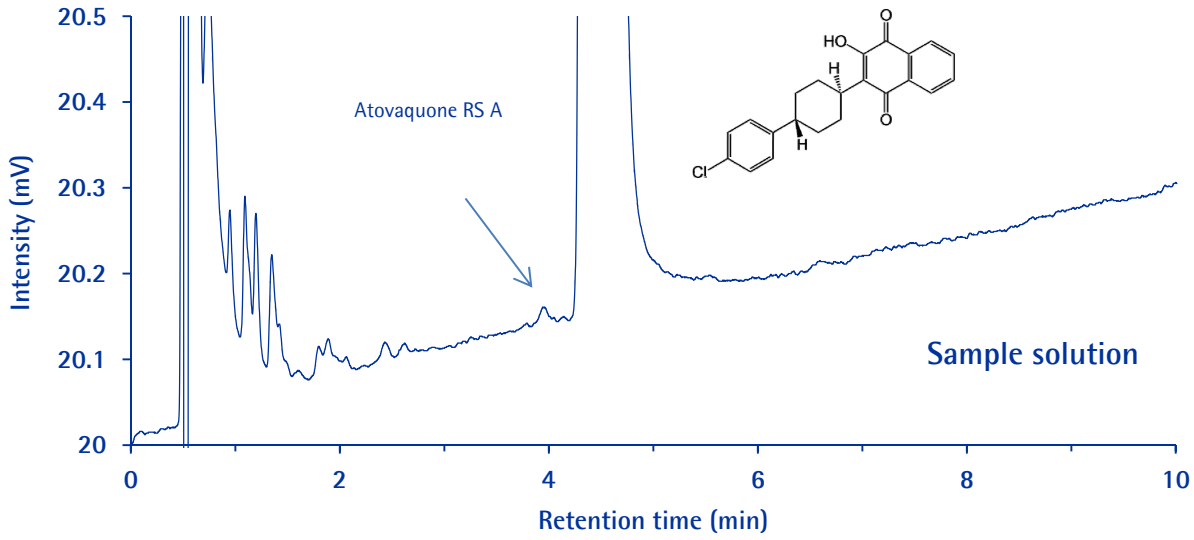


Figure 2. Chromatographic results of the atovaquone sample solution (0.09 mg/mL atovaquone from the sample stock solution) at 3.0 mL/min flow rate on a 100x4.6 mm id Chromolith® HighResolution RP-18 endcapped column. The system backpressure under these conditions is only 104 bar (1510 psi).

Chromatographic Data:

No.	Compound	Retention Time (min)	RRT	Tailing factor	Theoretical Plate
1	Atovaquone related compound A	3.93	0.88	-	-
2	Atovaquone	4.43	1.00	1.2	9466

As illustrated by Figure 2 and the performance table above, both the criteria for peak tailing (NMT 1.5), and the relative retention time (RRT) for Atovaquone related compound A and Atovaquone is met. Further details of this method can be found on page 28-30.

The method transfer is within allowed changes for partial revalidation.

United States Pharmacopoeia

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>

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

Performance Characteristics	Category 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.

Reference Standards

"*Reference Standards provided by the United States Pharmacopeial Convention (USP Reference Standards, or RS) are highly characterized specimens reflective of specified drugs and foods (drug substances, biologics, excipients, dietary supplements, food ingredients, impurities, degradation products, reagents, and performance verification standards). When approved as suitable for use as comparison standards for documentary tests or assays (i.e., as a monograph component) in the United States Pharmacopeia (USP) or National Formulary (NF), USP RS also assume official status and legal recognition in the United States. Assessment of the suitability for use in other applications rests with the user. Official USP RS are primary standards in jurisdictions that so recognize them as such and, when appropriate, are calibrated relative to international reference materials such as those provided by the World Health Organization.*"

USP Monograph Modernization

The United States Pharmacopeia (USP) develops and publishes monographs and general chapters that provide public quality standards for drugs, excipients, and dietary supplements in the United States Pharmacopeia and the National Formulary (USP–NF). USP has started a global initiative to modernize many of the existing monographs and is actively seeking industry collaborators to assist in the development of such monographs. The direct participation of the pharmaceutical industry, and other interested stakeholders in this program are encouraged to assist in providing updated public standards to strengthen the protection of public health. USP intends to modernize these monographs as soon as possible; either by traditional submission from a stakeholder or from USPs internal laboratory efforts. For more information, please contact the Standards Acquisition Department at stacq@usp.org.

Method Validation (Assay method)

The following pages introduces a procedure how to plan method validation per pharmacopoeial requirements.

Specificity test:

1. Use a blank solution to show no interference
2. Use placebo to demonstrate the lack of interference from excipients
3. Use spiked samples to show that all known related substances are resolved from each other
4. Use stressed samples with about 10 to 20% degradation are used to demonstrate the resolution among degradation products. Check the peak purity of the drug substance by using a photodiode array detector (PDA); e.g. purity angle is lower than the purity threshold.
5. Representative chromatograms should be provided with time scale and attenuation indicated

Linearity and Range:

From the calibration curve we can calculate the slope and from the slope we can calculate the limit of detection (LOD) and limit of quantitation (LOQ). In this example, the 100% concentration level corresponds to 75 ppm of main molecule, see table below.

Concentration (ppm)	% to concentration
112.5	150
90	120
75	**100**
60	80
37.5	50
18.75	25
7.5	10
3.75	5
0.75	1

LOQ Repeatability:

Good repeatability at LOQ level is a requirement and average value, the deviation from mean and the relative standard deviation in % should be calculated from minimum 10 injections, see table.

Injection Number	Area
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Average	
Standard Deviation (SD)	
Relative Standard deviation (RSD) %	

Accuracy and Recovery:

At least three different standard concentrations should be analyzed from final method sample concentrations; in this example and as in most cases for the LOQ level, 25% and 50 % level.
A sample set can be designed in the following way (using standard addition technique):

Sample Concentration (ppm)	Recovery level	Area
75	0 (means no addition to standard concentration 75 ppm)	
75	LOQ (means addition of LOQ concentration + 75 ppm)	
75	25 % (means addition of 25% level = 18.75 + 75 ppm)	
75	50 % (means addition of 50% level = 37.5 + 75 ppm)	
LOQ *(From calibration curve under linearity)*		
25% (means 18.75)		
50% (means 37.5)		

Recovery or accuracy can be calculated by the difference in area of pure sample and recovery standards and should be reported in percentage (%). The acceptable limit is 85-110 %.

Precision:

To determine the method precision multiple measurements of a defined sample should be carried out by the same analyst. A minimum of six (6) injections at the test concentration (6 times of a single batch) must be carried out, or to perform analysis at three (3) different concentration levels (80, 100, and120%) with three (3) repetitions each. The latter approach is a convenient way to also allow for method accuracy determination. For a monograph assay method the relative standard deviation should be better than 2.0% (RSD < 2.0%).

Intermediate Precision:

Ruggedness should be determined by analysis of same sample on multiple days, with multiple analysts, and multiple equipments. Repeat the method precision by different analyst in different equipment using different column lots on different days (three different column lots are generally recommended). The RSD should be within same level as for the method precision.
For a monograph assay method the relative standard deviation should be better than 2.0% (RSD < 2.0%).

Robustness:

To determine the method's capability to remain unaffected, small but deliberate variations in method parameters are carried out and where the following changes can be used as a recommendation.

1. Influence of variations of pH in a mobile phase (Changing buffer pH by +/- 0.2 units)
2. Influence of variations in mobile phase composition (Change in organic composition +/- 2.0%)
3. Test different columns (different lots and/or suppliers)
4. Temperature
5. Flow rate (+/- 0.2˚C)

The acceptance criteria are that the system suitability test (SST) parameters should pass for all the conditions and all known and unknown impurities shall be well separated from each other.

Validation Protocols (Related Substances)

A related substances method commonly have more stringent requirements, specifying relative retention time (RRT) and some time the relative response factors (RRF). From RRT, the retention time of the impurity can be identified and from RRF one may calculate the percentage of impurities present in the sample. As an example, let us look at the current Clarithromycin related substances method (USP37-NF32). The RRT and RRF given for Clarithromycin and 16 Clarithromycin impurities are given in the following table.

Name	RRT	RRF
Clarithromycin impurity I	0.38	1.0
Clarithromycin impurity A (Clarithromycin F)	0.42	1.0
Clarithromycin impurity J	0.63	1.0
Clarithromycin impurity L	0.74	1.0
Clarithromycin impurity B	0.79	1.0
Clarithromycin impurity M	0.81	1.0
Clarithromycin impurity C	0.89	1.0
Clarithromycin impurity D	0.96	1.0
Clarithromycin	**1.0**	-
Clarithromycin impurity N	1.15	1.0
Clarithromycin related compound A	1.27	1.0
Clarithromycin impurity F	1.33	1.0
Clarithromycin impurity P	1.35	1.0
Clarithromycin impurity O	1.38	1.0
Clarithromycin impurity K	1.59	1.0
Clarithromycin impurity G	1.72	3.7
Clarithromycin impurity H	1.82	6.7

The percentage of each impurity is calculated using the following formula:

Result = $(rU/rS)\times(CS/CU)\times(1/F)\times 100$
rU = peak response of any individual impurity (related substances; RS) from the Sample solution
rS = peak response of Clarithromycin from Standard solution 3
CS = concentration of USP Clarithromycin RS in Standard solution 3 (mg/mL)
CU = concentration of Clarithromycin in the Sample solution (mg/mL)
F = Relative response factor as mentioned in table

The validation of Clarithromycin related substances can be carried out using Standard Solution 3 *(specific details are given in the current monograph but not important for the general discussion here.)*, setting the Clarithromycin concentration as 100% concentration and following the same procedure as described in the assay. The concentration of any individual impurity should not be more than 0.4 % and total impurities NMT 3.5%.

Using then Standard solution 1 (Clarithromycin concentration is 1.5 mg/m L or 1500 ppm, the 0.4 % level (of 1500 ppm) correspond to 6 ppm. If any individual impurity standard is available then validation should be carried out using a 6 ppm concentration as 100% and repeat analysis as described in the assay.

Linearity and Range (see Assay procedure):
From the calibration curve we can calculate the slope and from the slope we can calculate the limit of detection (LOD) and limit of quantitation (LOQ). In this example, the 100% concentration level corresponds to 6 ppm of USP Clarithromycin RS.

LOQ Repeatability, Accuracy and Recovery:
For RS monograph method it possible to proceed as described in the assay method. The difference is that the relative standard deviation must not be more than 10% for the related substances, and the concentration of Clarithromycin RS sample solutions are much lower, i.e. 6 ppm, LOQ, 25% and 50%.

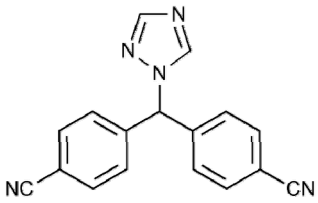
Injection Number	Area
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Average	
Standard Deviation (SD)	
Relative Standard deviation (RSD) %	

Sample Concentration (ppm)	Recovery level	Area
6.0	0 (means no addition to standard concentration 6 ppm)	
6.0	LOQ (means addition of LOQ concentration + 6 ppm)	
6.0	25 % (means addition of 25% level = 1.5 + 6 ppm)	
6.0	50 % (means addition of 50% level = 37.5 + 6 ppm)	
LOQ *(From calibration curve under linearity)*		
25% (means 1.5)		
50% (means 3.0)		

On the following pages, a complete method validation is presented for the **Letrozole and Related Substances monograph method (USP).**

Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped



Letrozole

Letrozole is an oral non-steroidal aromatase inhibitor for the treatment of hormonally-responsive breast cancer after surgery. Estrogens are produced by the conversion of androgens through the activity of the aromatase enzyme. Estrogens then bind to an estrogen receptor, which causes cells to divide. Letrozole prevents the aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit. The action is specific, and Letrozole does not reduce production of mineralo- or corticosteroids. The commercial trade name of Letrozole is Femara.

The current USP monograph method for Letrozole and related substances specifies use of a 125x4.6 mm column with L1 (RP-18/ODS) (5 µm) packing as stationary phase, and with identical experimental conditions as described in the assay method. System suitability requirements for related substances are provided by means of resolution between Letrozole and Letrozole related substance A and should not be less than 2.0, as well as a relative retention time RRT of 0.67 Letrozole related substance A, 1.0 for Letrozole and 2.4 for 4,4',4''-Methanetriyltribenzonitrile. The tailing factor should be 0.8-1.5 for Letrozole.

The following pages illustrate a complete procedure for the method validation within limits of allowed changes. All acceptance critera are met for the Letrozole and related substances method by using the identical matched column; 125x4.6 mm Purospher® STAR RP-18 endcapped (5 µm) column for Letrozole analysis.

Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 125x4.6 — 1.51914.0001

Injection: 20 µL

Detection: UV 230 nm

Cell: 10 µL

Flow Rate: 1.0 mL/min

Mobile Phase: A: Water
B: Acetonitrile

Gradient: See table

Time (min)	A (%)	B (%)
0.01	70	30
25.0	30	70
26.0	70	20
30.0	70	30

Temperature: 25°C

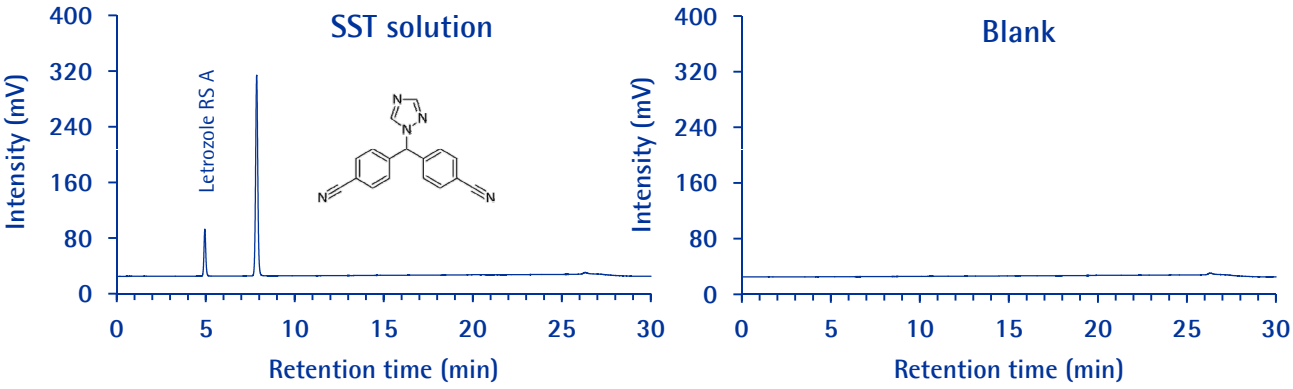
Diluent: Acetonitrile and water; 3:7 (v/v)

SST solution: 2 µg/mL of Letrozole Related Compound A and 10 µg/mL of Letrozole in Diluent

Standard solution: 1 µg/mL of USP Letrozole in Diluent.

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

Pressure Drop: 72 - 28 Bar (1044 - 410 psi)



Chromatographic Data :

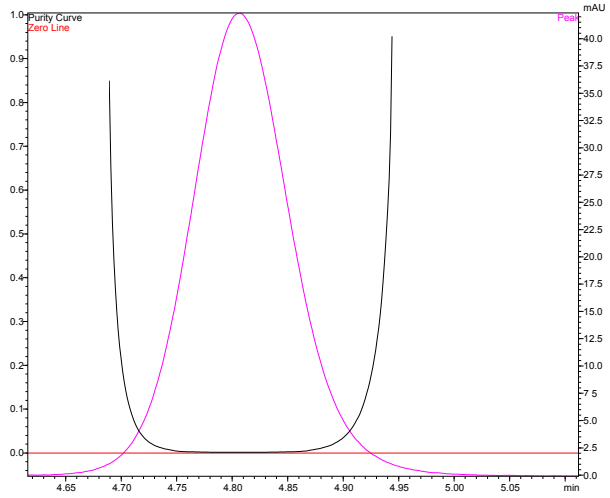
No.	Compound	Retention Time (min)	RRT	Resolution	Theoretical Plate	Assymetry
1	Letrozole related compound A	4.9	0.62		11641	1.10
2	Letrozole	7.9	1.0	14.3	20133	1.07

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

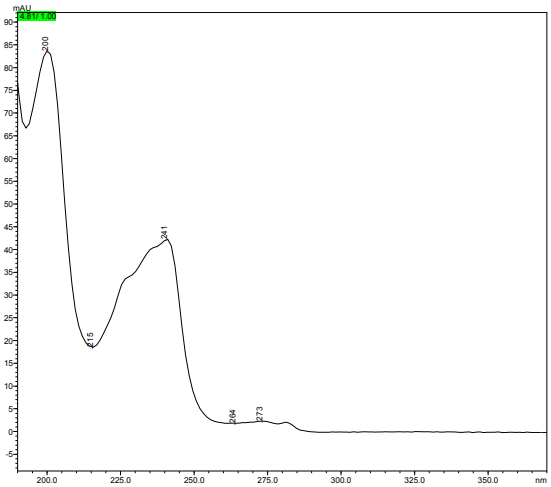
Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

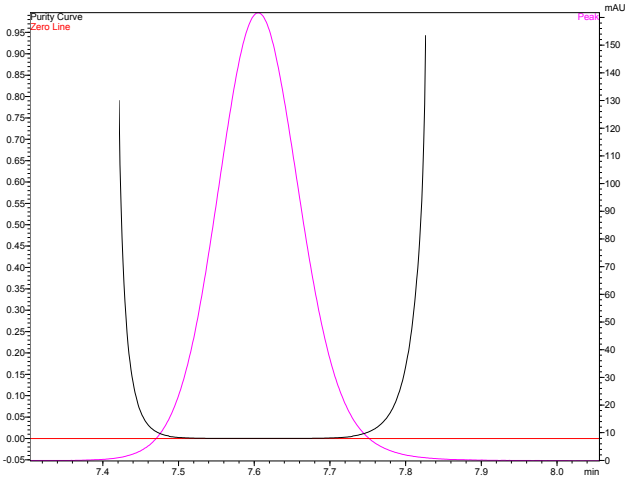
Peak Purity curve: Letrozole RS A



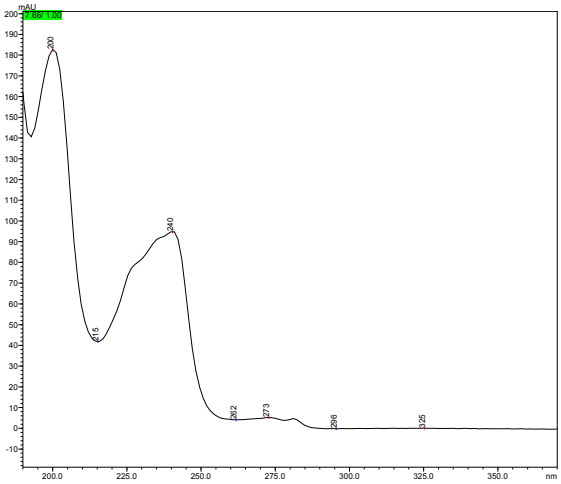
UV spectra: Letrozole RS A



Peak Purity curve: Letrozole



UV spectra: Letrozole



Letrozole and Related Substances (USP)

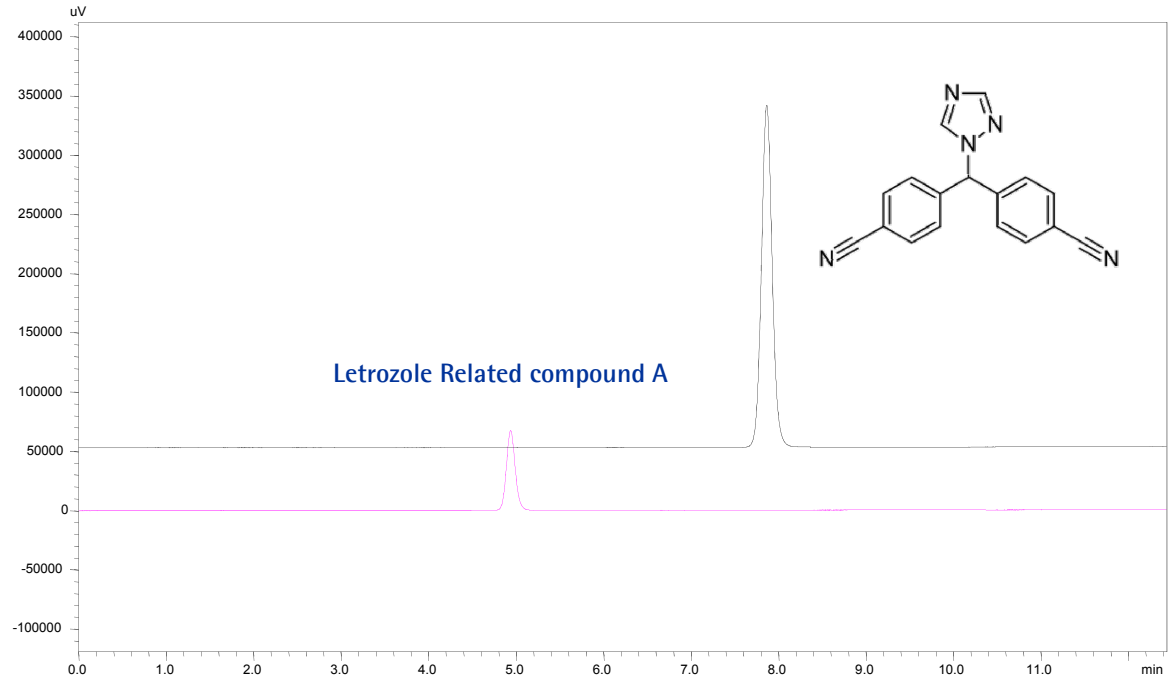
Purospher® STAR RP-18 endcapped

Method Validation

Following parameters have been checked during the validation of Letrozole

- Specificity
- Precision and Robustness
- Linearity
- Limit of Detection (LOD) and Limit of Quantitation (LOQ)
- LOQ repeatability

Specificity :
Each individual component of the sample has been analysed using the same conditions.
Not a single component or blank has any interference on each other.
The method stands specific using the Purospher® STAR RP-18 endcapped column.



Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Precision:

The retention time precision achieved for both cases is < 0.5 % RSD.
The area precision achieved for both the cases is < 1 % RSD.

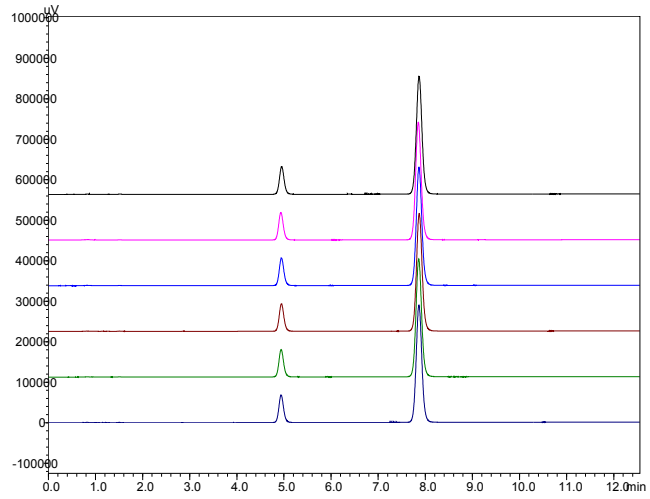


Table I: System Precision

	Retention Time (min)		Area	
Injection	RS A	Letrozole	RS A	Letrozole
1	4.98	7.90	474836	2457649
2	4.97	7.89	474364	2458516
3	4.97	7.89	474541	2450935
4	4.96	7.87	474747	2449480
5	4.95	7.86	474431	2450486
6	4.93	7.84	473816	2451989
Average	4.96	7.874	474456	2453176
Std. Dev.	0.02	0.023	362	3895
% RSD	0.40	0.29	0.08	0.16

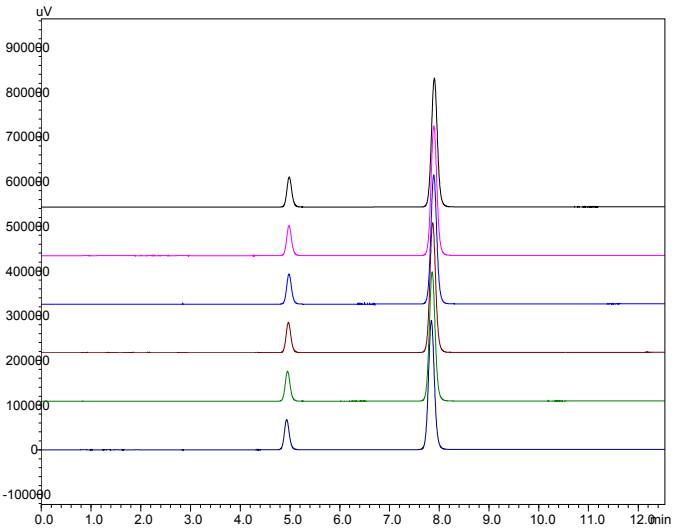


Table II: Method Precision

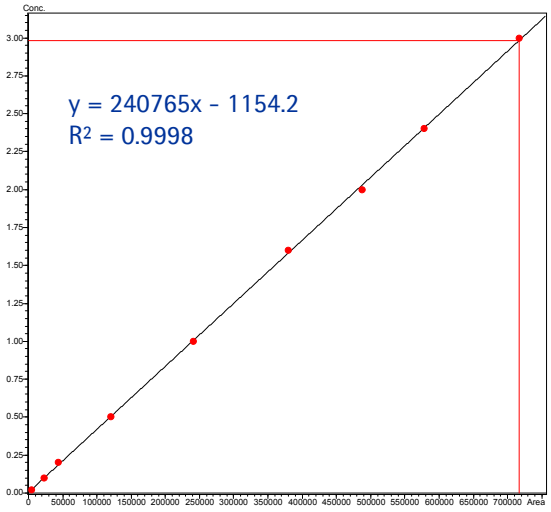
	Retention Time (min)		Area	
Injection	RS A	Letrozole	RS A	Letrozole
1	4.93	7.85	478983	2473247
2	4.95	7.87	479259	2478348
3	4.95	7.87	480187	2479408
4	4.94	7.86	479478	2479989
5	4.94	7.86	480206	2477724
6	4.95	7.87	478293	2486492
Average	4.94	7.863	480206	2479201
Std. Dev.	<0.01	<0.01	3294	4294
% RSD	0.12	0.09	0.69	0.17

Letrozole and Related Substances (USP)

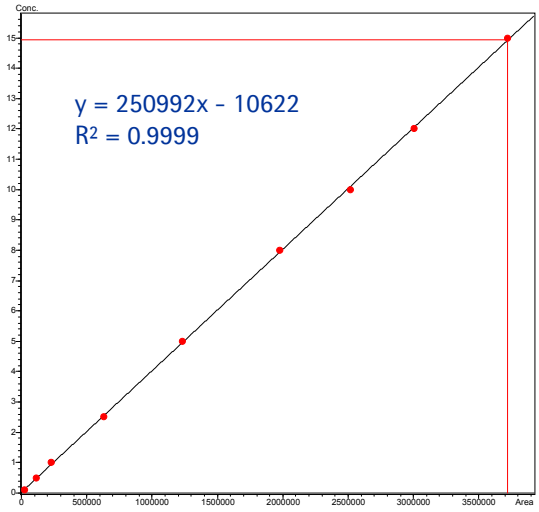
Purospher® STAR RP-18 endcapped

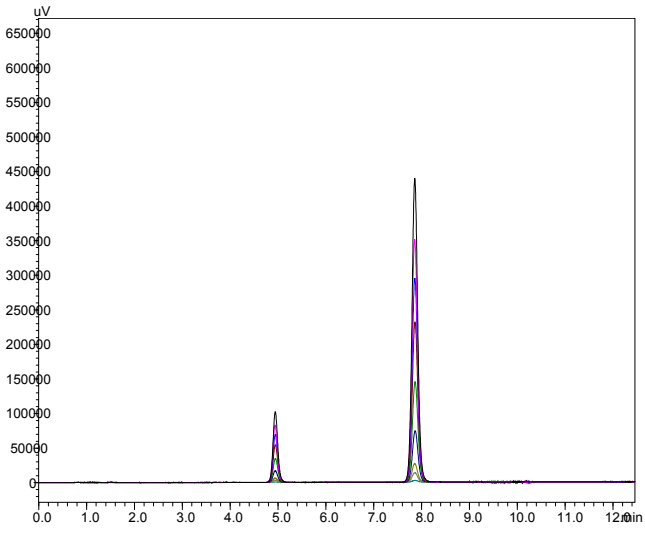
Linearity:

Letrozole Related Substance A



Letrozole





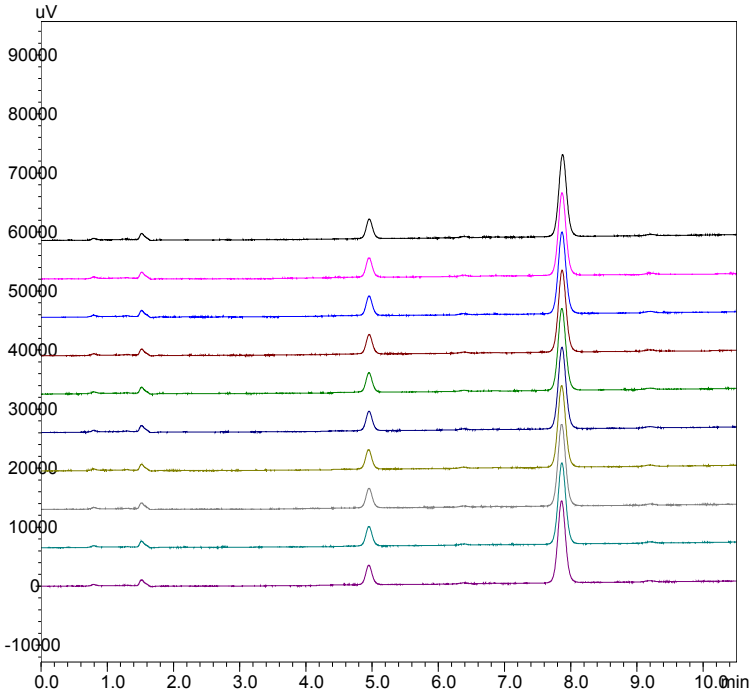
Letrozole RS A		Letrozole	
Conc (ppm)	Area	Conc (ppm)	Area
0.02	4394	0.1	22557
0.1	22630	0.5	116715
0.2	43882	1.0	229577
0.5	120464	2.5	631260
1.0	240666	5.0	1231279
1.6	380178	8.0	1976461
2.0	487146	10.0	2519018
2.4	578138	12.0	3001958
3.0	717196	15.0	3722293

LOD for Related Compound A 0.03 ppm
LOQ for Related Compound A 0.1 ppm

Letrozole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

LOQ repeatability Related Substance A:



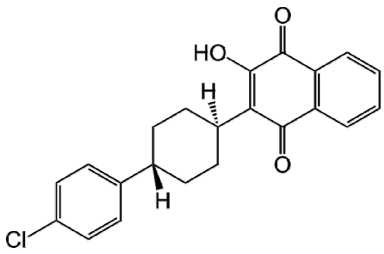
Injection	Area
1	22718
2	22826
3	22825
4	22730
5	22799
6	22498
7	22622
8	22346
9	22291
10	22555
Average	22621
Std. Dev	194.54
% RSD	0.86 %

Relative standard deviation (RSD in %) for Letrozole Related Substance A at LOQ level 0.86%

Conclusion:

The analytical HPLC method for Letrozole related substance has been validated and it passed all method validation criteria with the Purospher® STAR RP-18 endcapped column.

Atovaquone Oral Suspension (USP)



Atovaquone belongs to the class of naphthoquinones; a hydroxy-1,4-naphthoquinone, an analog of ubiquinone, with antipneumocystic activity. It is manufactured in the US in the liquid form, or oral suspension, under the brand name Mepron.

The current USP monograph for Atovaquone oral suspension specifies the use of a L1 (RP-18) column with 125x4.6 mm geometry (no particle size mentioned), used at a flow rate of 3.0 mL/min.

At this flow rate, with normal particulate columns (5 µm), the back pressure over the HPLC system will be very high on a fresh column, and only after some analyses it goes beyond the sustainable backpressure limit and causes failure in analysis.

Of these reasons we have transferred the current method to a 100x4.6 mm id monolithic column, i.e. a Chromolith® HighResolution RP-18 endcapped column, where the back pressure is only around 100 bar (1440 psi) under given experimental conditions. A pressure that any HPLC system easily can accommodate, and thereby provide robust and reliable results over time.

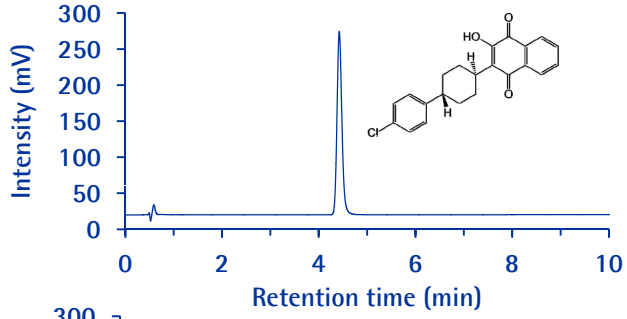
The method meet all acceptance criteria with good reproducibility for the specified sample solution per current monograph, and will not require a complete re-validation for acceptance.

Atovaquone Oral Suspension (USP)

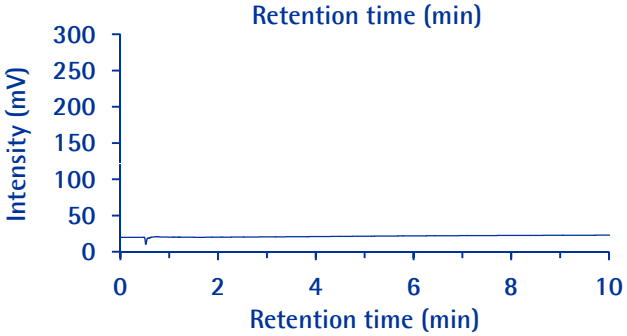
Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	3.0 mL/min	
Mobile Phase:	Acetonitrile, methanol, water, and phosphoric acid; 480:160:360:5 (v/v/v/v)	
Temperature:	30°C	
Diluent:	0.1 M methanolic sodium hydroxide	
SST Solution:	Nominally 3 mg/mL from a known volume of well-mixed oral suspension	
Standard Solution:	Not less than (NLT) 750 mg of atovaquone prepared as following procedure: In an appropriately sized, low-actinic volumetric flask, add 20% volume of water, swirl for 5 min, add 60% volume of 0.1 M methanolic sodium hydroxide, and sonicate for 15 min. Allow to cool, and dilute with 0.1 M methanolic sodium hydroxide to volume. Immediately filter a 20-ml portion, discarding the first 5 mL of the filtrate.	
Sample solution:	0.09 mg/mL of atovaquone from the clear filtrate of the Sample stock solution. Transfer to an appropriately sized, low-actinic volumetric flask, and dilute with a mixture of methanol and water (1:1) to volume. **Minimize exposure of this solution to light.**	
Pressure Drop:	104 Bar (1510 psi)	



Sample solution



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Suitability requirement: NMT 1.5

Chromatographic Data :

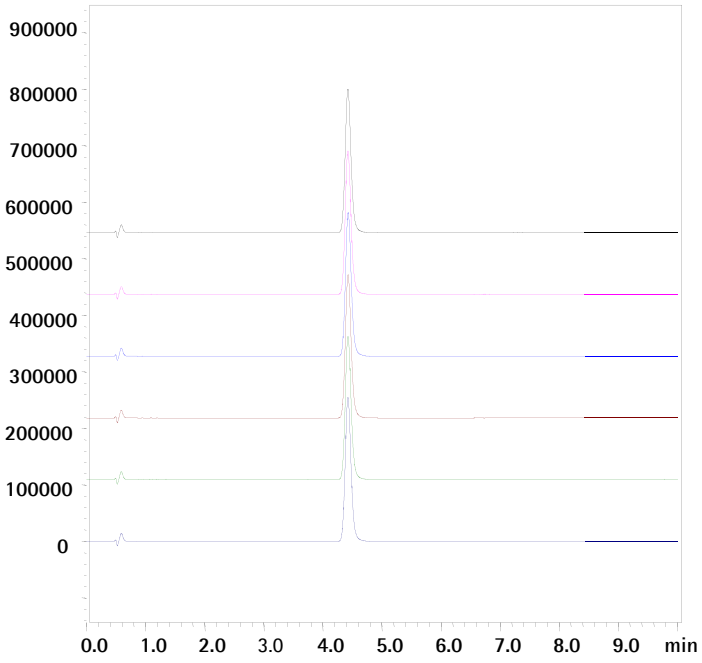
No.	Compound	Retention Time (min)	Tailing factor	Theoretical Plate
1	Atovaquone	4.4	1.2	9466

Atovaquone Oral Suspension (USP)

Chromolith® HighResolution RP-18 endcapped

Repeatability Data

The relative standard deviation (RSD) for six repeated analyses of the sample solution (0.09 mg/mL of atovaquone) were excellent with far better precission than the specified suitability requirement (NMT 2.0%).

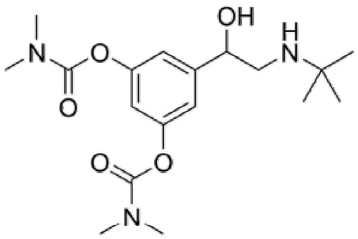


Injection Number	Retention time	Area
1	4.42	1786281
2	4.43	1784126
3	4.43	1784598
4	4.42	1786092
5	4.42	1785619
6	4.43	1788002
Average	4.43	1785786
Standard Deviation	0.00	1374
RSD (%)	0.04	0.08

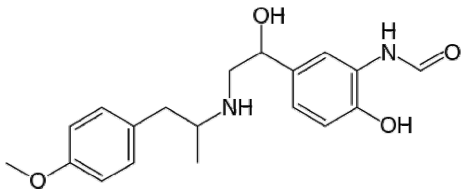
Recommended chemicals and reagents to reproduce this alternative method

Product	Name of the Chemical	Article Number
1	ortho-Phosphoric acid 85% for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1.00573
2	Sodium hydroxide pellets for analysis (max. 0.02% K) EMSURE® ACS, Reag. Ph Eur	1.06469
3	Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.00030
4	Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.06007

Bambuterol and Related Substances (EP)



Bambuterol



Formoterol

Bambuterol is a long acting beta-adrenoceptor agonist (LABA) used in the treatment of asthma. It is also a prodrug of terbutaline. Commercially, bambuterol is marketed as Bambec and Oxeol.

Formoterol or eformoterol is a long-acting β2 agonist used in the management of asthma and chronic obstructive pulmonary disease (COPD). It is marketed in four forms: a dry-powder inhaler, a metered-dose inhaler, an oral tablet, and an inhalation solution, under various trade names including Foradil/Foradile, Oxeze/Oxis, and (with Budenoside) Symbicort, Atock, Atimos, and Perforomist.

The current EP monograph for Bambuterol and related substances specifies the use of a 150x4.6 mm column with base-deactivated octadecylsilyl (ODS or RP-18) silica gel for chromatography R (5 µm). The total run-time must be 1.5 times the retention time of bambuterol, with the following retention times; formoterol = about 7 min; bambuterol = about 9 min. If necessary, the composition of the mobile phase can be adjusted; increase the content of phosphate buffer to increase the retention time to meet a chromatographic resolution of minimum 5.0 between the bambuterol and formoterol peaks.

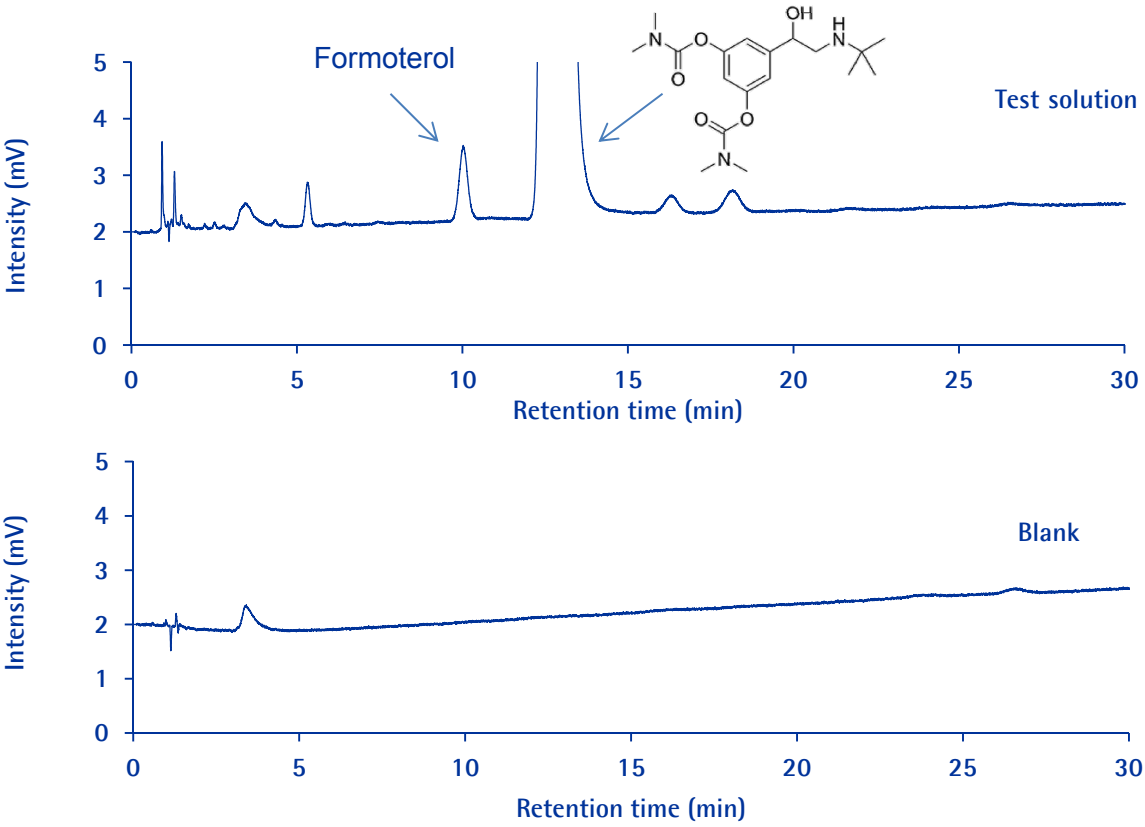
The following pages illustrate that acceptance critera are being met, and that a Purospher® STAR RP-18 endcapped (5 µm), Hibar® RT 150x4.6 mm column is an excellent alternative for Bambuterol analysis.

Bambuterol and Related Substances (EP)

Purospher® STAR RP-18 endcapped

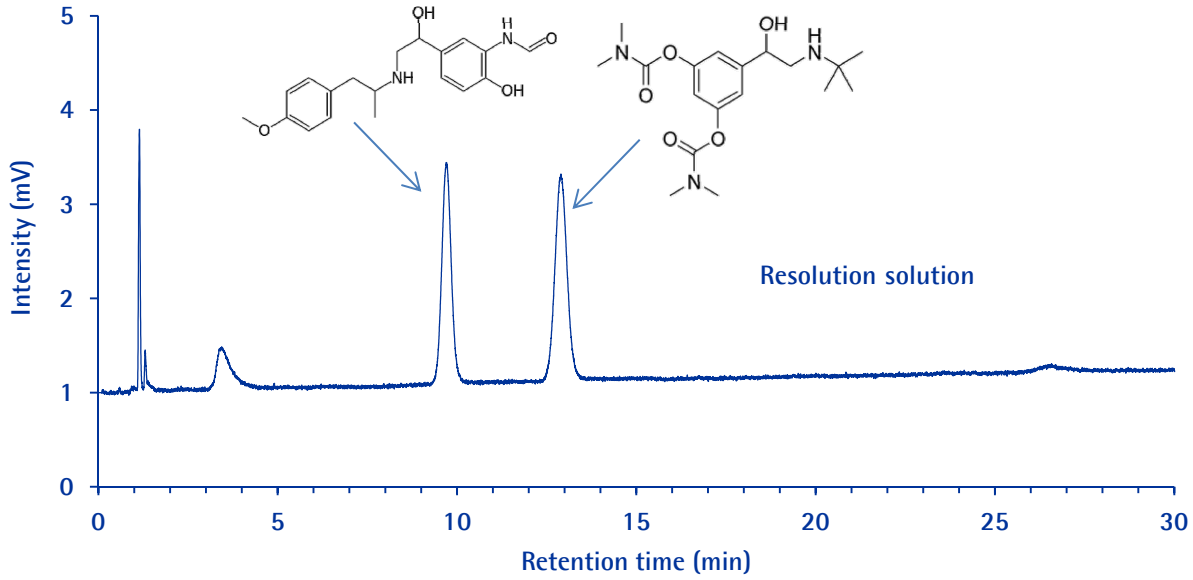
Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5 µm), Hibar® RT 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	UV, 214 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Dissolve 6.90 g of sodium di-hydrogen phosphate monohydrate in water and dilute to 1000 mL with water. Adjust to pH 3.0 with a 50 g/L solution of dilute phosphoric acid. Dissolve 1.3 g of sodium octanesulfonate in 430 mL of a mixture of 25 volumes of acetonitrile and 75 volumes of methanol and 570 ml of buffer solution.	
Temperature:	Ambient	
Diluent:	Mobile phase	
Test Solution:	5.0 mg Bambuterol in 10 ml mobile phase.	
Resolution Solution:	Dissolve 1.0 mg of formoterol fumarate dihydrate in the mobile phase and dilute to 10.0 mL with the mobile phase. Mix 0.8 mL of this solution with 0.4 mL of the test solution and dilute to 100.0 mL with the mobile phase.	
Pressure Drop:	224 Bar (3248 psi)	



Bambuterol and Related Substances (EP)

Purospher® STAR RP-18 endcapped

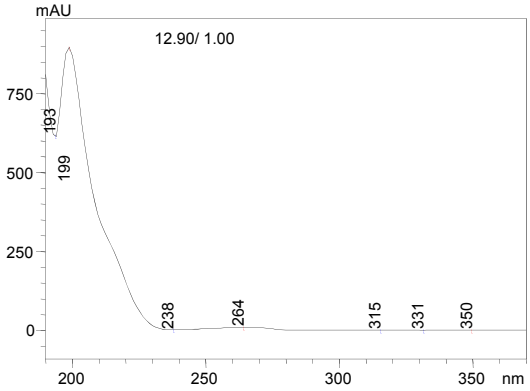


Suitability requirement: NLT 5.0

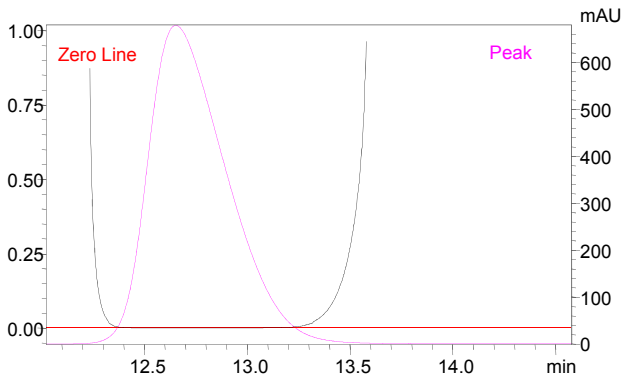
Chromatographic Data:

No.	Compound	Retention Time (min)	Resolution	RRT
1	Formoterol	9.7		0.75
2	Bambuterol	12.9	6.2	1.00

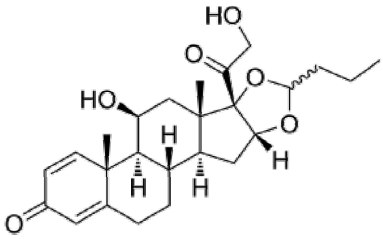
UV spectra: Bambuterol



Peak purity curve - Bambuterol



Budesonide and Related Substances (EP)



Budesonide

Budesonide is a glucocorticoid steroid for the treatment of asthma, COPD and non-infectious rhinitis (including hay fever and other allergies), and for treatment and prevention of nasal polyposis.
In addition, it is used for Crohn's disease (inflammatory bowel disease).

A new extended-release formulation of budesonide called "Uceris" has been recently approved (2013) by the United States FDA for ulcerative colitis.

The current EP monograph method for Budesonide and related substances specifies the use of a 150x4.6 mm column with and end-capped octadecylsilyl (ODS or RP-18) silica gel for chromatography R (3 µm). The relative retention with reference to budesonide epimer B (retention time = about 17 min); impurity A = about 0.1; epimers of impurity D = about 0.63 and 0.67; impurity L = about 0.95; epimers of impurity G = about 1.2 and 1.3; epimers of impurity K = about 2.9 and 3.0. The system suitability criteria also specify a minimum resolution of 1.5 between the 2 principal peaks (budesonide epimers A and B) in the chromatogram.

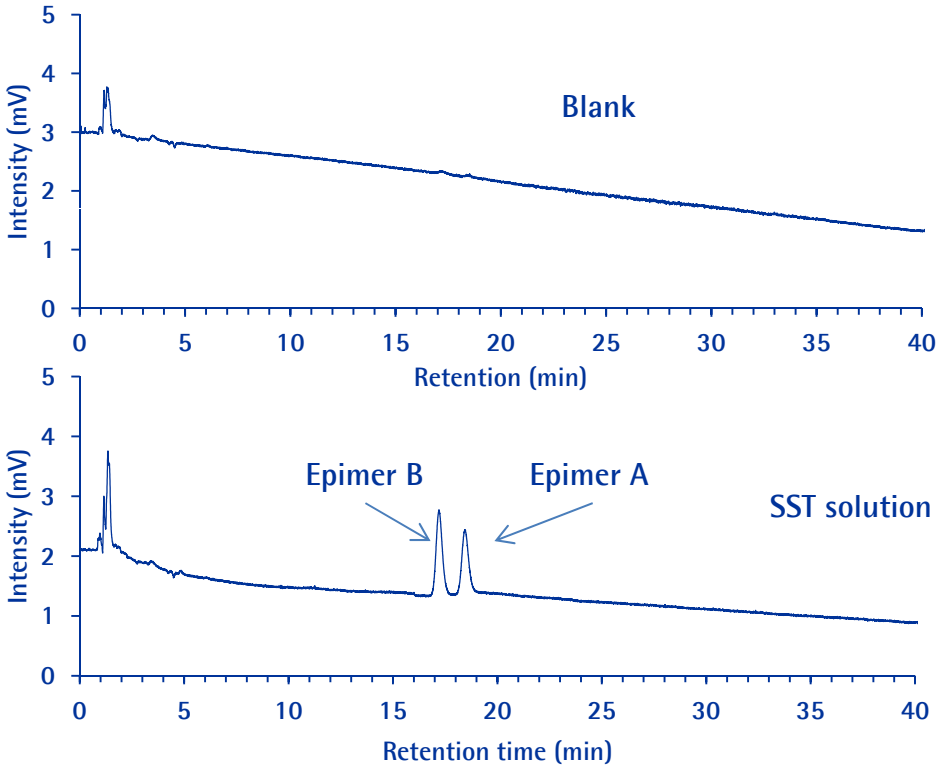
The following pages illustrate that the acceptance criteras are being met despite Impurity A and epimers of impurity K are not identified in the given chromatogram (not present in tested sample). Thus using a Purospher® STAR RP-18 endcapped (3 µm), Hibar® RT 150x4.6 mm column it is possible to comply with the given requirement for the analysis of Budesonide and related substances.

Budesonide and Related Substances (EP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

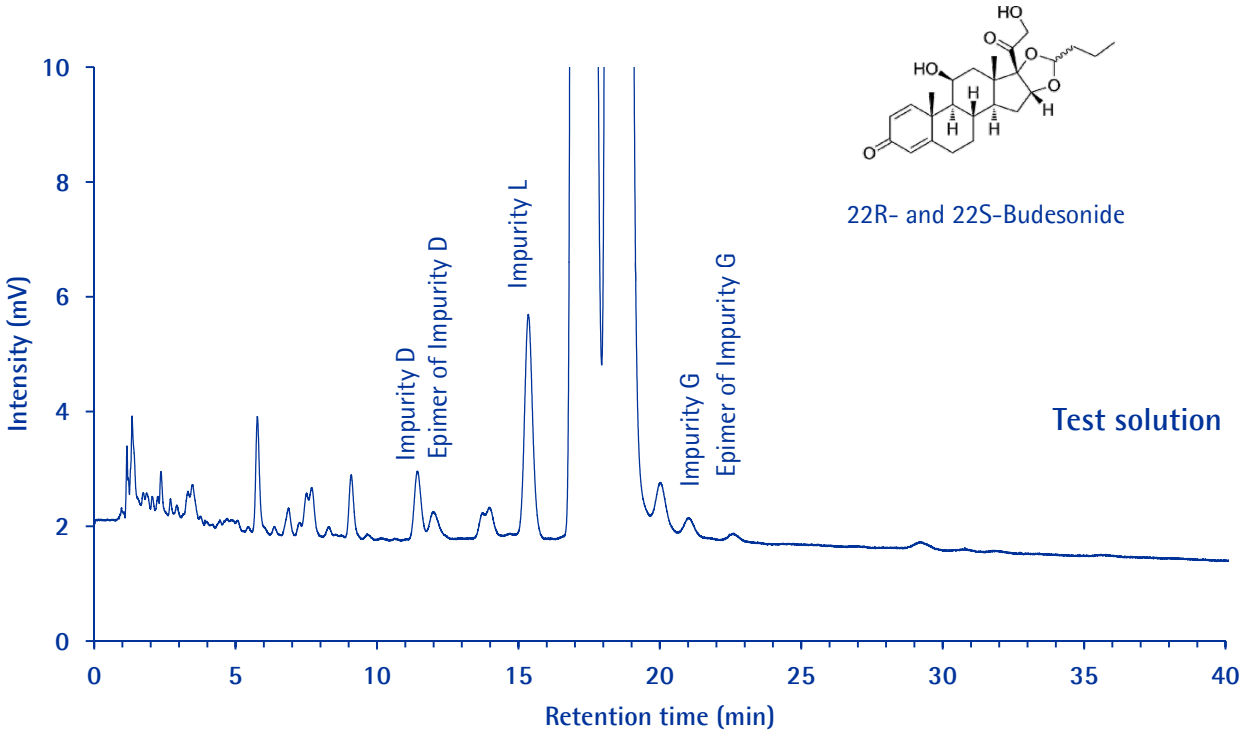
Column:	Purospher® STAR RP-18 endcapped (3 µm) Hibar®150x4.6 mm	1.50470.0001
Injection:	20 µL	
Detection:	UV, 240 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: to 900 mL of a 4 g/L solution of sodium dihydrogen phosphate , add 100 mL of a 2.5 g/L solution of phosphoric acid . Adjust the pH if necessary. A: Anhydrous ethanol , acetonitrile, phosphate buffer solution pH 3.2; 2:32:68 (v/v/v) B: Acetonitrile, phosphate buffer solution pH 3.2 R; 50:50 (v/v)	
Gradient:	See table	
Temperature:	50°C	
Diluent:	Acetonitrile R, phosphate buffer solution pH 3.2; 32:68 (v/v)	
Test Solution:	50 mg of the substance to be examined in 15 mL of acetonitrile and dilute to final volume (50 mL) with phosphate buffer solution pH 3.2.	
Resolution Solution:	Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.	
Pressure Drop:	182 - 162 Bar (2639 - 2349 psi)	



Time (min)	A (%)	B (%)
0.0	100	0
38.0	100	0
50.0	0	100
60.0	0	100
65.0	100	0
70.0	100	0

Budesonide and Related Substances (EP)

Purospher® STAR RP-18 endcapped



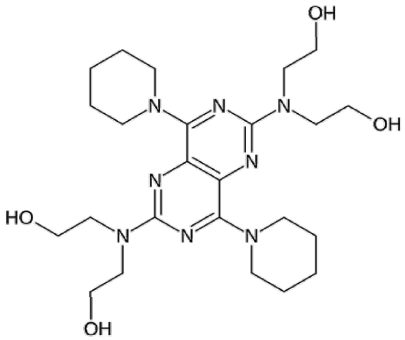
In this sample neither impurity A, nor the epimers of impurity K could be found!

Suitability requirement: NLT 1.5

Chromatographic Data:

No.	Compound	Retention Time (min)	Resolution	RRT
1	Impurity D	11.4		0.66
2	Epimer of Impurity D	12.0		0.69
3	Impurity L	15.4		0.90
4	Budesonide Epimer B	17.2		1.00
5	Budesonide Epimer A	18.4	2.2	1.07
6	Impurity G	21.0		1.22
7	Epimer of Impurity G	22.6		1.31

Dipyridamole and Related Substances (EP)



Dipyridamole

Dipyridamole inhibits thrombus formation when given chronically and causes vasodilatation when given at high doses over a short time. Common tradename is Persantine.

The current EP monograph method for Dipyridamole and related substances specifies the use of a 100x4.0 mm column with end-capped octadecylsilyl (ODS or RP-18) silica gel for chromatography R (5 µm). The relative retention with reference to dipyridamole (retention time = about 8 min); impurity B = about 0.2; impurity F = about 0.3; impurity D = about 0.9; impurity E = about 1.3; impurity C = about 1.6; impurity A = about 2.2.– The system suitability criteria also specify a minimum resolution of 2.0 between the peaks due to impurity D and dipyridamole.

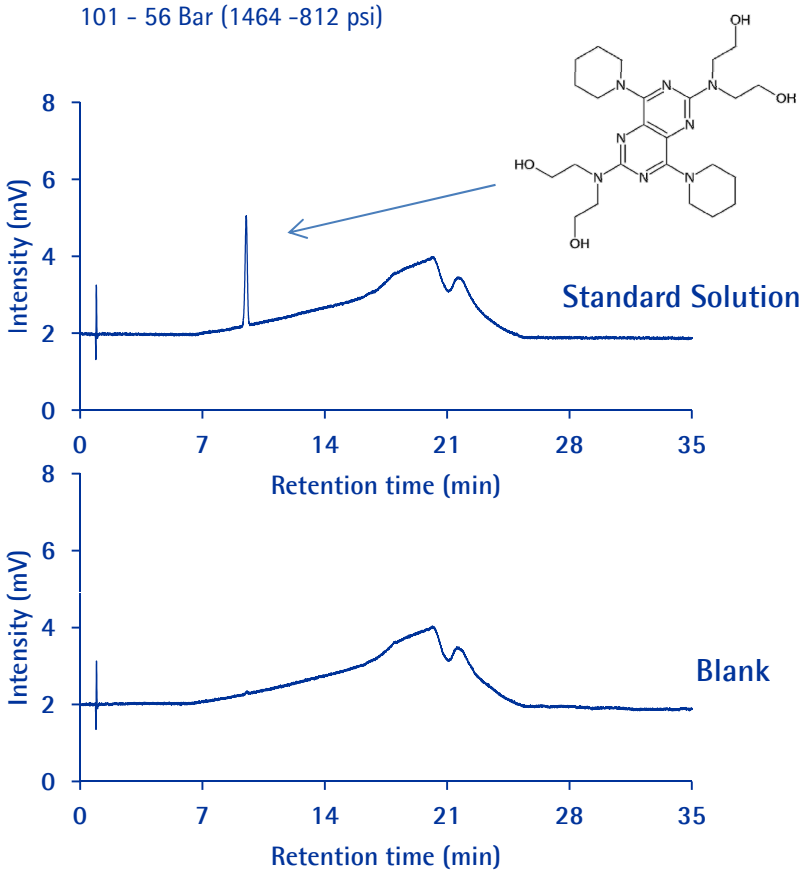
The current EP method specifies the use of a 100x4.0 mm column with 5 µm RP-18 stationary phase, but we used a 100x4.6 mm column instead (allowed change). Due to the large column tube volume we added another six minutes for column re-equilibration which explains why total method run-time is six minutes longer.

The following pages illustrate that the acceptance critera are being met and it is possible to use a 100x4.6 mm Purospher® STAR RP-18 endcapped (5 µm) column to comply with the given requirement.

Dipyridamole and Related Substances (EP)

Purospher® STAR RP-18 endcapped

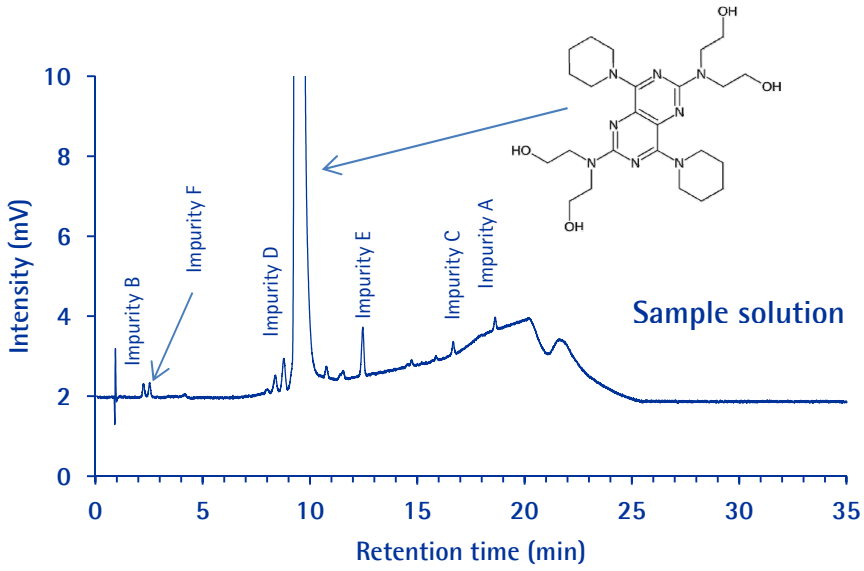
Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 100x4.6 mm	1.50622
Injection:	5 µL	
Detection:	UV 295 nm	
Cell:	10 µL	
Flow Rate:	1.2 mL/min	
Mobile Phase:	A: dissolve 1.0 g of potassium dihydrogen phosphate R in 900 mL of to water adjust to pH 7.0 with 0.5 M sodium hydroxide and dilute 1000 mL with water. B: Methanol	
Gradient:	See table	
Temperature:	45°C, Sample cooler at 10°C	
Diluent:	Methanol	
Sample Solution:	Dissolve 0.100 g of the substance to be examined in methanol and dilute to 50 mL with the same.	
Reference Solution:	Dilute 1.0 mL of the test solution to 100.0 mL with methanol. Dilute 1.0 mL of this solution to 10.0 mL with methanol	
Pressure Drop:	101 - 56 Bar (1464 -812 psi)	



Time (min)	A (%)	B (%)
0.0	40	60
5.0	40	60
19.0	5	95
24.0	40	60
35.0	40	60

Dipyridamole and Related Substances (EP)

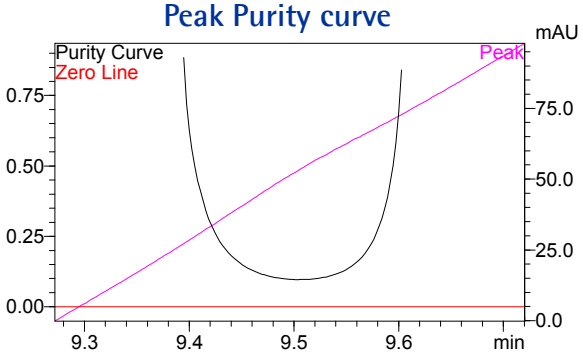
Purospher® STAR RP-18 endcapped



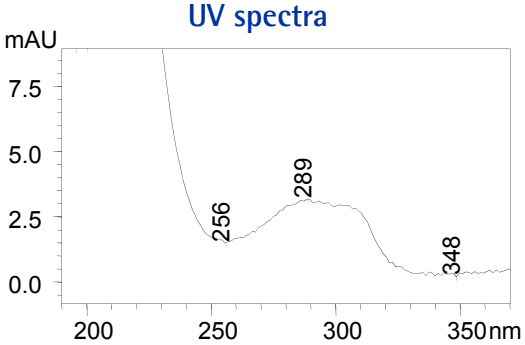
Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	Observed RRT	RRT per EP
1	Impurity B	2.2		0.23	About 0.2
2	Impurity F	2.5		0.26	About 0.3
3	Impurity D	8.8		0.93	About 0.9
4	Dipyridamole	9.5	3.3	1.00	About 1.0
5	Impurity E	12.5		1.31	About 1.3
6	Impurity C	16.7		1.75	About 1.6
7	Impurity A	18.6		1.96	About 2.2

Peak Purity curve



UV spectra



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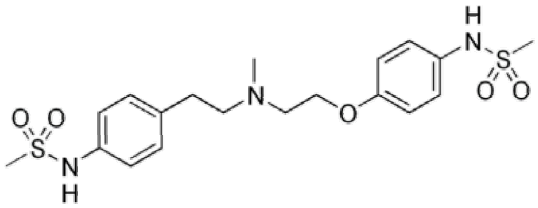
chromatography@merckgroup.com

Dofetilide and Related Substances (USP)



Dofetilide

Dofetilide is a class III antiarrhythmic agent. It is marketed under the trade name Tikosyn.
Due to the pro-arrhythmic potential, it is only available by prescription by physicians who have undergone specific training in the risks of treatment with dofetilide.

The current USP monograph method for Dofetilide and related substances specifies the use of a 150x3.9 mm column with and L1 (ODS or RP-18) packing having 5 µm particle size. System suitability requirements; a resolution of NLT 5.0 between dofetilide and dofetilide related compound A; a column efficiency of NLT 35,000 theoretical plates for the dofetilide peak; and with a tailing factor of NMT 1.5 for the dofetilide peak (using the system suitability solution).

We have redeveloped this monograph method using a monolithic Chromolith® HighResolution RP-18 endcapped column and the following pages illustrate that the all acceptance critera are being met and the new method provide excellent performance at very low system pressure.

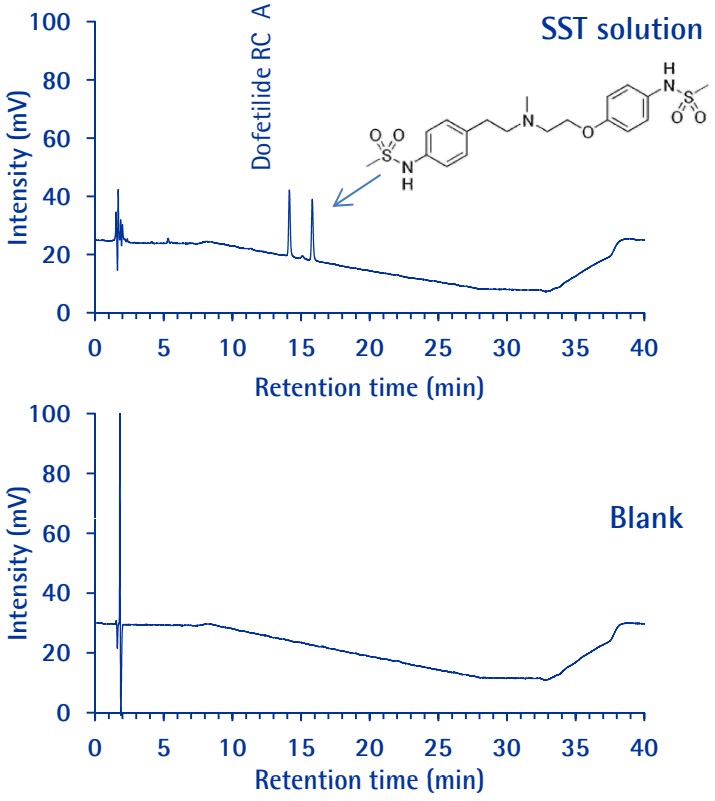
Using a monolithic column over a particulate counterpart, additional column life-time can be expected due to the more robust stationary phase backbone. In addtion, a monolithic column exhibit lower backpressure and thus less wear on HPLC system (extra safety and lower maintenance cost).
In summary, this means an overall lower cost per analysis.

Dofetilide and Related Substances (USP)

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV 230 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	A: 0.78 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 5.0 ± 0.1 B: Methanol	
Gradient:	See table	
Temperature:	30°C	
Diluent:	Acetonitrile and Buffer; 3:22 (v/v)	
SST Solution:	1.25 µg/mL each of Dofetilide and Dofetilide Related Compound A in diluent	
Standard Solution:	1.25 µg/mL of USP Dofetilide in Diluent	
Sample soltion	0.25 mg/mL of Dofetilide in Diluent	
Pressure Drop:	52-65 Bar (754 - 943 psi)	



Time (min)	A (%)	B (%)
0.0	88	12
5.0	88	12
25.0	70	30
30.0	70	30
35.0	88	12
40.0	88	12

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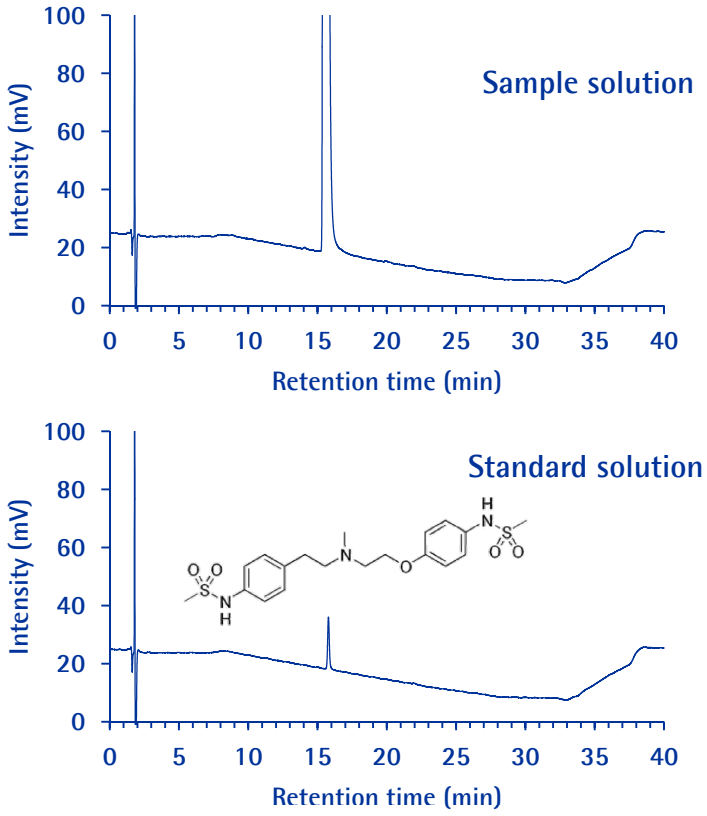
chromatography@merckgroup.com

Dofetilide and Related Substances (USP)

Chromolith® HighResolution RP-18 endcapped



With the use of a Chromolith® HighResolution column you can meet the system suitability without changing any parameter besides a reduction in injection volume from 50 to 20 µL.

New method would require revalidation though because of specification in monograph of column with particle size.

Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	Theoretical Plate
1	Dofetilide Related compound A	14.2		69968
2	Dofetilide	15.8	7.7	83075

Parameter	USP requirement	Observed value
Resolution	5.0	7.7
Theoretical plate for Dofetilide peak	35000	83075
Tailing factor for Dofetilide peak	NMT 1.5	1.1

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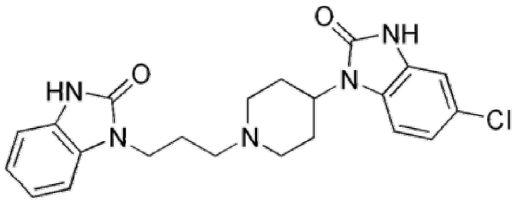
Domperidone and Related Substances (EP)



Domperidone

Domperidone is a peripheral, specific blocker of dopamine receptors. Domperidone is given in order to relieve nausea and vomiting; to increase the transit of food through the stomach (as a prokinetic agent through increase in gastrointestinal peristalsis); and to increase lactation (breast milk production) by release of prolactin. Common trade names are: Motilium, Motillium, Motinorm Costi, Nomit and Molax.

The current EP monograph method for Domperidone and related substances specifies use of a 100x4.6 mm column with base-deactivated octadecylsilyl (ODS or RP-18) silica gel for chromatography R (3 µm) as stationary phase. System suitability requirements specify the relative retention with reference to domperidone (retention time = about 6.5 min): impurity A = about 0.4; impurity B = about 0.65; impurity C = about 0.7; droperidol = about 1.1; impurity D = about 1.15; impurity E = about 1.2; impurity F = about 1.3.

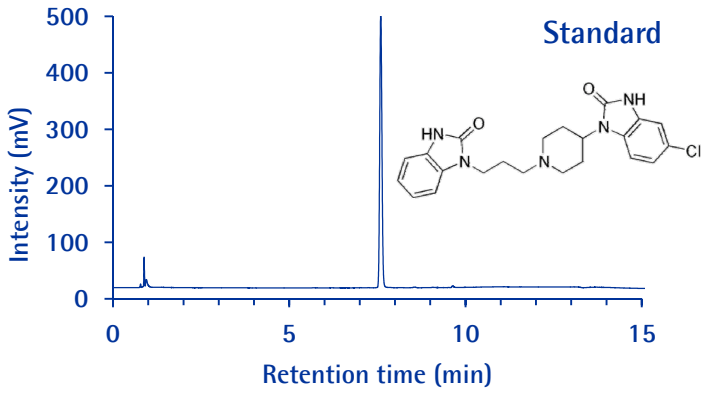
The following pages illustrate that the acceptance critera are being met for Domperidone by following the current EP monograph. Thus using a Purospher® STAR RP-18 endcapped (3 µm), Hibar® RT 100x4.6 mm column it is possible comply with the requirement for Domperidone and related substances analysis.

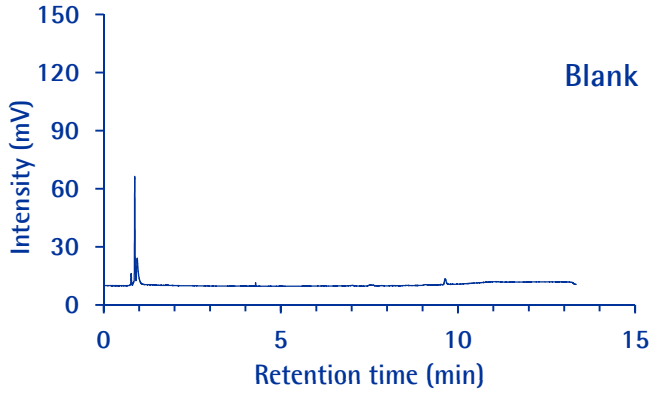
Domperidine and Related Substances (EP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions:

Column:	Purospher® STAR RP-18 endcapped (3µm) Hibar® RT 100x4.6 HPLC column	1.50469.0001
Injection:	10 µL	
Detection:	UV, 284 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Solution A: 5 g/L solution of ammonium acetate Solution B: Methanol	
Gradient:	See table	
Temperature:	25 °C	
Diluent:	Dimethylformamide	
Refrence Solution:	Dilute 1.0 mL of the test solution to 100.0 mL with dimethylformamide. Dilute 5.0 mL of this solution to 20.0 mL with dimethylformamide.	
Test Solution:	Dissolve 0.10 g of the domperidone in dimethylformamide and dilute to 10.0 mL with the same solvent.	
Pressure Drop:	260 - 104 Bar (3770 - 1508 psi)	

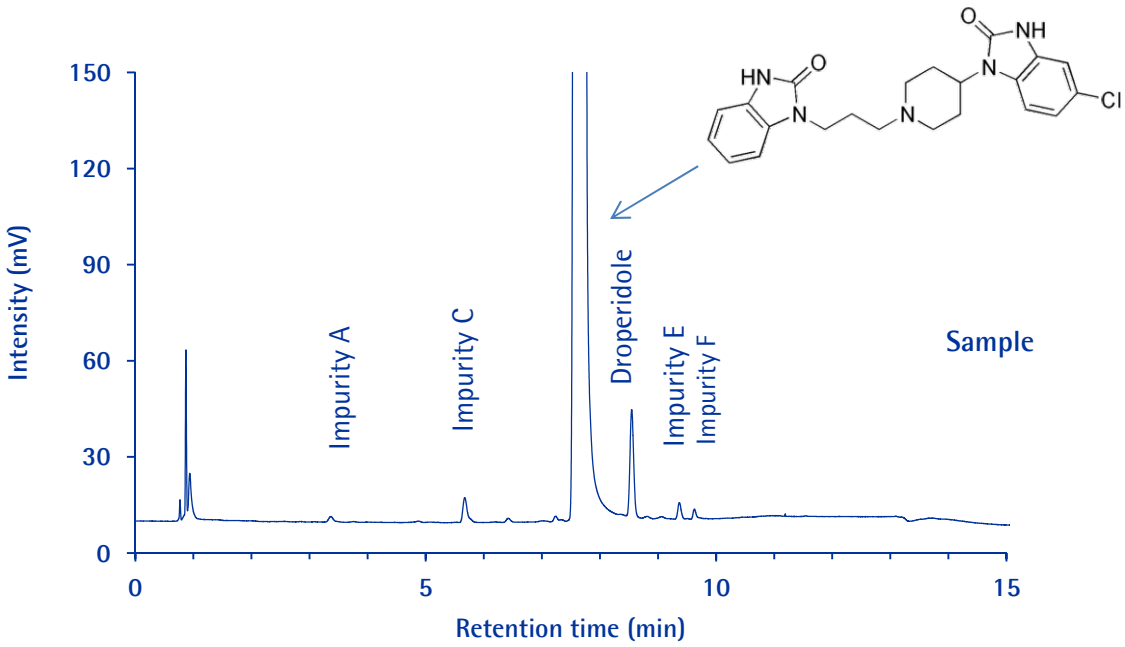




Time (min)	A (%)	B (%)
0.01	70	30
10.0	0	100
12.0	0	100
15.0	70	30
20.0	70	30

Domperidine and Related Substances (EP)

Purospher® STAR RP-18 endcapped

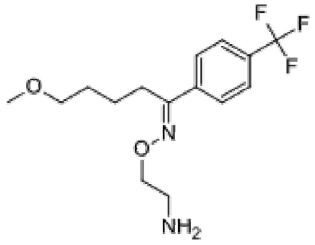


No impurity B (RRT of about 0.65) nor impurity D (RRT of about1.15) could be identified in this sample

Chromatographic Data : Sample

No.	Compound	Retention Time (min)	Resolution	Observed RRT	RRT Specification (EP)
1	Impurity A	3.4		0.44	About 0.4
2	Impurity C	5.7		0.75	About 0.7
3	Domperidone	7.6		1.00	
4	Droperidole	8.5	6.0	1.11	About 1.1
5	Impurity E	9.4		1.23	About 1.2
6	Impurity F	9.6		1.27	About 1.3

Fluvoxamine and Related Substances (USP)



Fluvoxamine

Fluvoxamine is a medication which functions as a selective serotonin reuptake inhibitor (SSRI) and σ1 receptor agonist. Fluvoxamine is used primarily for the treatment of obsessive-compulsive disorder (OCD), and is also used to treat major depressive disorder (MDD), and anxiety disorders such as panic disorder and posttraumatic stress disorder (PTSD).Fluvoxamine CR (controlled release) is approved to treat social anxiety disorder. Common commercial brand names: Floxyfral, Luvox, Fevarin.

The current USP monograph method for Fluvoxamine maleate and related substances specifies the use of a 250x4.6 mm column with L7 (RP-8) packing as stationary phase (no particle size mentioned), and with identical experimental conditions as described in the assay method. System suitability requirements for related substances are provided by means of relative retention time (RRT) for identified and known impurites, see tabulated impurites in chromatography performance table. In addition, for the assay method, the column efficiency should be not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

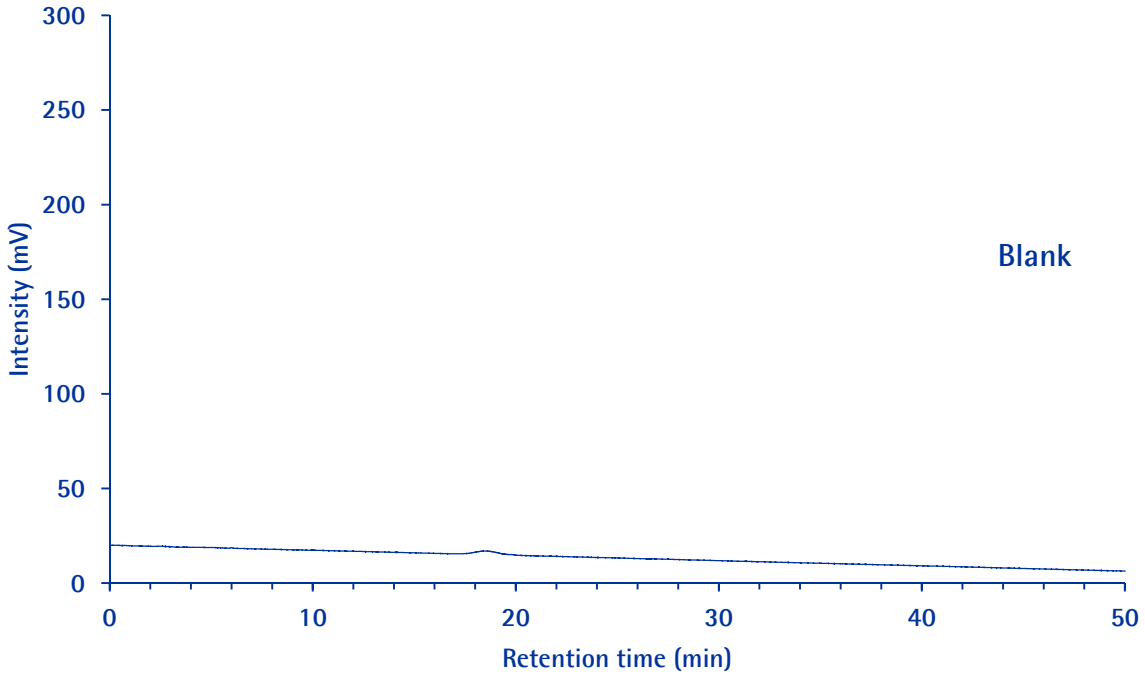
The following pages illustrate that the acceptance critera are being met for the Fluvoxamine maleate and related substances methods by following the current USP monograph and using identical matched column. Using a 250x4.6 mm Purospher® STAR RP-8 endcapped (5 µm) column it is possible to comply with the requirement for Fluvoxamine maleate and related substances analysis.

Fluvoxamine and Related Substances (USP)

Purospher® STAR RP-8 endcapped

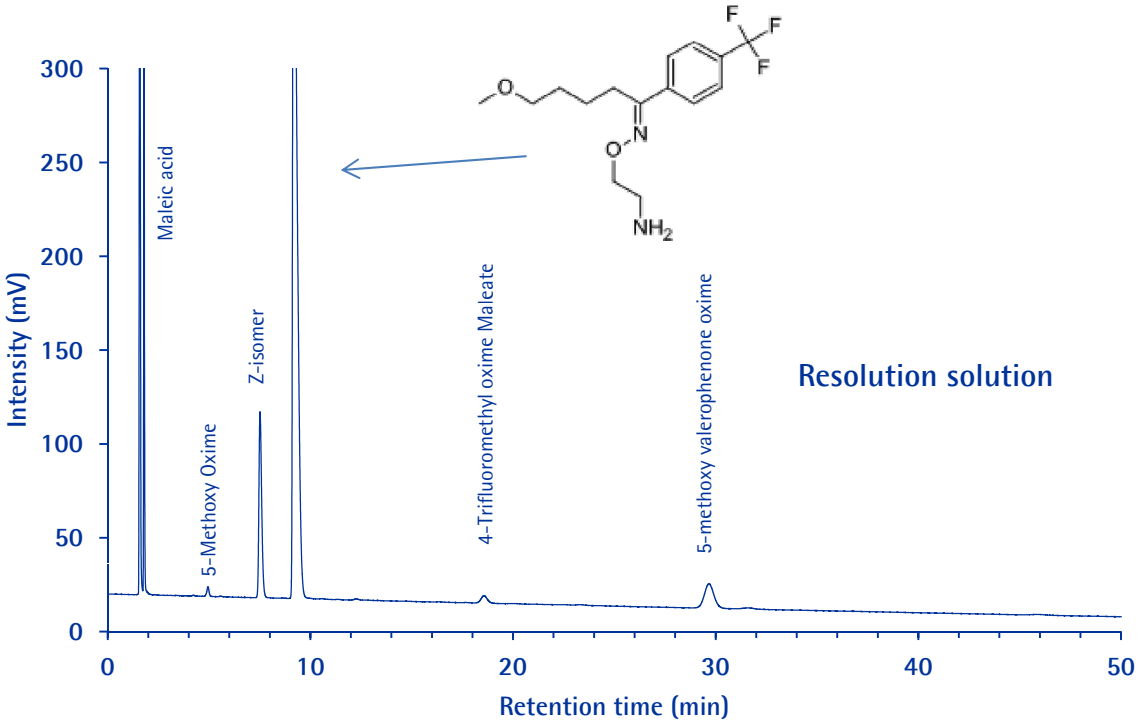
Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (5µm) Hibar® 250x4.6 mm	1.51454.0001
Injection:	20 µL	
Detection:	UV 234 nm	
Cell:	10 µL	
Flow Rate:	1.7 mL/min	
Mobile Phase:	Buffer: dissolve about 5 g of 1-pentanesulfonic acid sodium salt and 0.7 g of monobasic potassium phosphate in 620 mL of water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05. Mix buffer and acetonitrile 62:38 (v/v)	
Temperature:	40 °C	
Diluent:	Mobile phase	
Resolution Solution:	Transfer about 6 mg of Fluvoxamine Maleate to a 50-mL volumetric flask. Heat the sample at 120 for 10 minutes. Cool down to room temperature, add 3.0 mL of 0.1 N hydrochloric acid. Heat the solution in a water bath for 10 minutes. Cool down to room temperature, add 50 mg of Fluvoxamine Maleate, and dissolve in 25 mL of Mobile phase. Dilute with Mobile phase to volume, and mix.	
Pressure Drop:	179 Bar (2596 psi)	



Fluvoxamine and Related Substances (USP)

Purospher® STAR RP-8 endcapped



Chromatographic Data : Sample

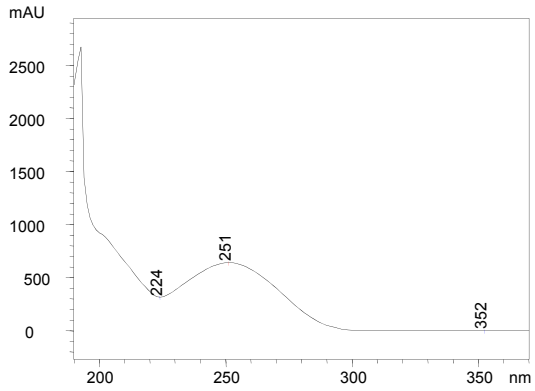
No.	Compound	Retention Time (min)	Resolution	Observed RRT	Approx RRT USP guideline
1	Maleic acid	1.6		0.17	0.19
2	5-Methoxy Oxime	4.9		0.54	0.50
3	Z- Isomer	7.5	11.6	0.82	0.79
4	Fluvoxamine	9.2	5.1	1.00	1.0
5	4-Trifluoromethyl oxime Maleate	18.6		2.02	2.00
6	5-methoxy valerophenone oxime	29.7		3.23	3.45

In the analyzed samples neither:
5-Methoxy-4¢-(trifluoromethyl)valerophenone(E)-O-(2-aminoethyl)aminoethyl oxime maleate (RRT 0.67);
4¢-(Trifluoromethyl)valerophenone(E)-O-2-(2-aminoethyl)aminoethyl oxime maleate (RRT 1.18);
(E)-O-2-(2-Aminoethyl)-4-(trifluoromethyl)- -phenylacetophenone oxime maleate (RRT 1.74) nor
5-Methoxy-4¢-(trifluoromethyl)valerophenone ketone (RRT 4.2) could be found!

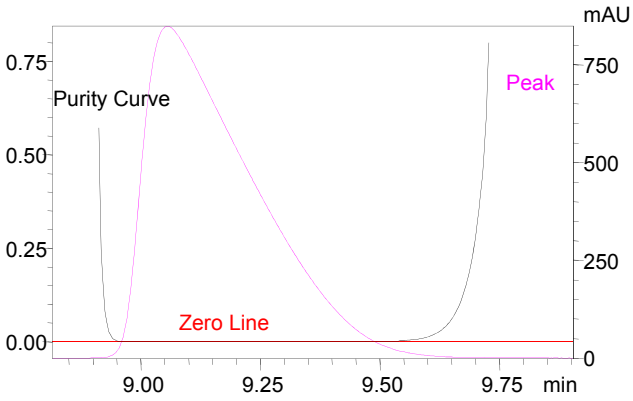
Fluvoxamine and Related Substances (USP)

Purospher® STAR RP-8 endcapped

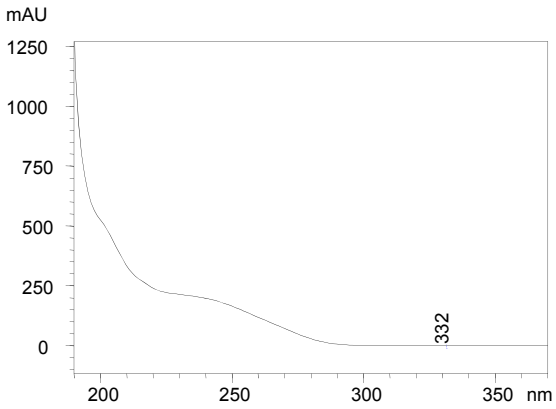
UV spectra: Fluvoxamine



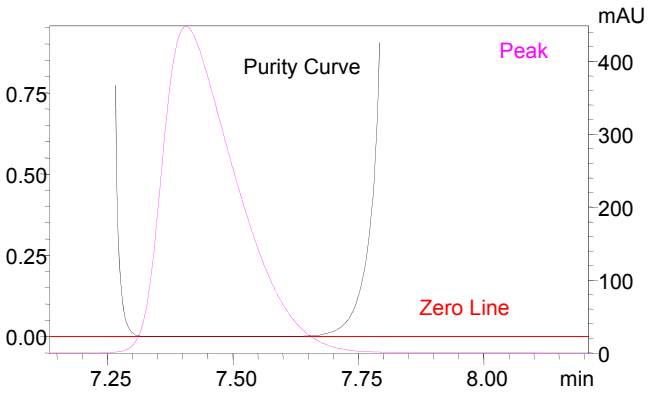
Peak purity curve: Fluvoxamine



UV spectra: Z-isomer

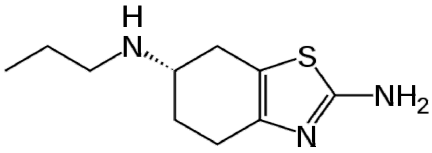


Peak purity curve: Z-isomer



Pramipexole and Related Substances (USP)

Purospher® STAR RP-18 endcapped



Pramipexole

Pramipexole is a dopamine agonist used for treating Parkinson's disease (PD) and restless legs syndrome (RLS). Common brand names: Mirapex, Mirapexin, Sifrol.

The current USP monograph method for Olanzapine and related substances as well as for the assay method specifies th use of a 150x4.6 mm column with 5 µm L1 (RP-18) packing as stationary phase. System suitability requirements for related substances are; Resolution: NLT 6.0 between pramipexole related compound A and pramipexole; and a tailing factor NMT 2.0 for pramipexole. In addition, in the assay method the relative retention times for pramipexole related compound A and pramipexole are specified about 0.7 and 1.0, respectively.

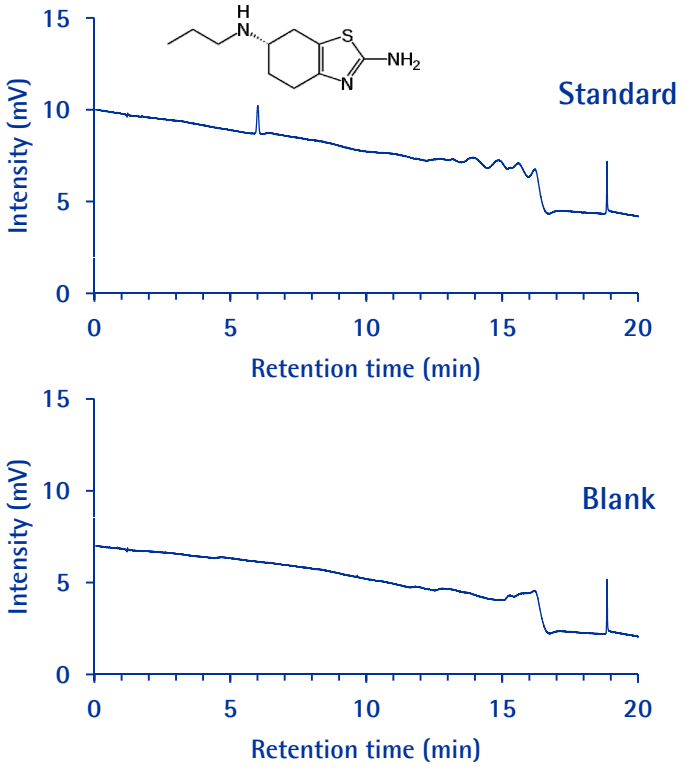
The following pages illustrate that the acceptance critera are met for both the Pramipexole and related substances as well as the assay method by following the current USP monograph using a 150x4.6 mm Purospher® STAR RP-18 endcapped (5 µm) column.

Pramipexole and Related Substances (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

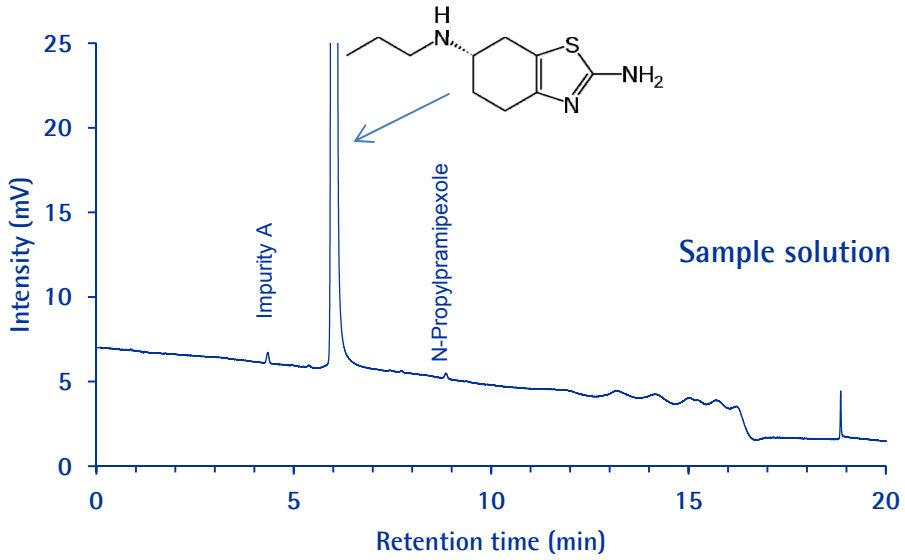
Column:	Purospher® STAR RP-18 endcapped (5 µm) Hibar® 150x4.6 mm	1.51455.0001
Injection:	5 µL	
Detection:	Shimadzu Prominence, UV 264 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Solution A: Dissolve 9.1 g of potassium dihydrogen phosphate and 5.0 g of sodium 1- octanesulfonate monohydrate in 1 L of water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05. Solution B: Acetonitrile and Solution A; 1:1 (v/v)	
Gradient:	See table	
Temperature:	45 °C	
Diluent:	Acetonitrile and Solution A; 1:4 (v/v)	
Resolution Solution:	1.5 µg/mL of USP Pramipexole Dihydrochloride in Diluent	
Sample solution:	1.5 mg/mL of Pramipexole Dihydrochloride in Diluent	
Pressure Drop:	209 - 215 Bar (3030 - 3117 psi)	



Time (min)	A (%)	B (%)
0.0	60	40
15.0	20	80
15.1	60	40
20.0	60	40

Pramipexole and Related Substances (USP)

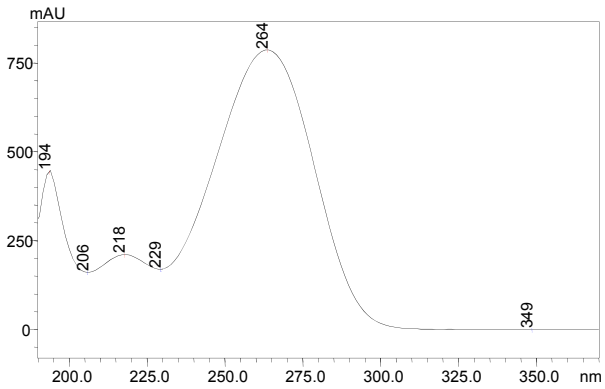
Purospher® STAR RP-18 endcapped



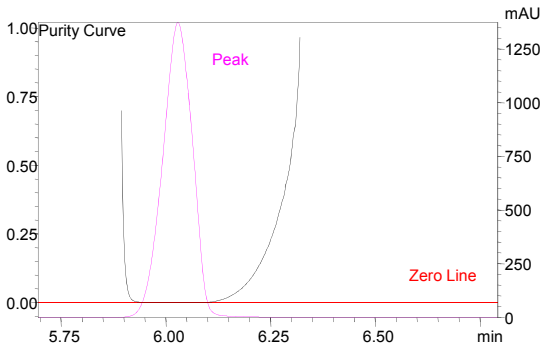
Chromatographic Data : Sample

No.	Compound	Retention Time (min)	Resolution	Tailing Factor	RRT
1	Impurity A	4.3			0.71
2	Pramipexole	6.0	13.9	1.00	1.00
3	N-Propylpramipexole	8.8			1.47

UV spectra - Pramipexole



Peak purity curve –Pramipexole



From HPLC to UHPLC

A transfer of HPLC methods to UHPLC requires scaling down from bigger to smaller inner diameter (e.g. 4.6 →2.1 mm i.d.) and from long to short columns (e.g. 250/150 to 100 or 50 mm length) in addition to the reduction of particle sizes (from e.g. 5 µm to 2 µm). To ensure equivalent chromatographic separation, it is also necessary to scale the flow rate, injection volume and the gradient parameters.

Adjusting the column length

The first step is to determine the appropriate column length in order to maintain the same separation. Keeping the same column length while decreasing the particle size will increase the number of theoretical plates as well as the backpressure. Therefore, when decreasing particle size, column length can be shortened without losing resolution.

$$\text{Column length } L_2 = L_1 \times dp_2 / dp_1$$

L_1 - HPLC column length
L_2 - UHPLC column length
dp_1 - HPLC particle size
dp_2 - UHPLC particle size

Scaling the flow rate

Decreasing the internal diameter of the column (e.g. from 4.6 to 2.1 mm) requires recalculating column flow rate in order to maintain same linear velocity. Linear velocity is defined as the distance which mobile phase travels over time (cm/min), whereas flow rate is the volume of mobile phase that travels over time (mL/min). To maintain the same linear velocity through a column with a smaller internal diameter, the flow rate must be decreased proportionally to the column internal diameter according to the equation below.

$$\text{Flow rate } f_2 = f_1 \times (d_2)^2 / (d_1)^2$$

f_1 - HPLC flow rate
f_2 - UHPLC flow rate (mL/min)
d_1 - HPLC column ID
d_2 - UHPLC column ID (mm)

Scaling the injection volume

Decreasing the column internal diameter and length, decreases the overall column volume and sample capacity. Therefore, we must alter the injection volume. Please note that since overall column volume has decreased, it is more important to match the sample solvent to the starting mobile phase composition. Mismatched sample solvents can cause irreproducible retention times, efficiencies, and even changes in selectivity. If using a larger injection volume than calculated, check for peak abnormalities and irreproducibility that could result from phase overload.

$$\text{Injection volume } V_2 = V_1 \times (d_2^2/d_1^2) \times (L_2 / L_1)$$

V_1 - HPLC Injection volume
V_2 - UHPLC Injection volume
d_1 - HPLC column ID
d_2 - UHPLC column ID (mm)
L_1 - HPLC column length
L_2 - UHPLC column length

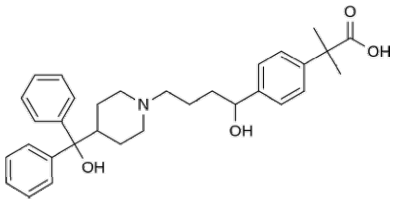
Adjusting gradient time

When an analytical method is scaled down, the time program of the gradient also needs to be scaled down to keep the gradient volume the same.

$$\text{Time: } t_2 = t_1 \times (f_1/f_2) \times (d_2^2/d_1^2) \times (L_2 / L_1)$$

t_1 - HPLC time
t_2 - UHPLC time
f_1 - HPLC flow rate
f_2 - UHPLC flow rate (mL/min)
L_1 - HPLC column length
L_2 - UHPLC column length

Fexofenadine and Related Substances (USP)



Fexofenadine

Fexofenadine is an antihistamine pharmaceutical drug used in the treatment of allergy symptoms, such as hay fever, nasal congestion, and urticaria. Common trade names are: Allegra, Fexidine, Telfast, Fastofen, Tilfur, Vifas, Telfexo, Allerfexo.

The current USP monograph method for Fexofenadine and related substances specifies the use of a 250x4.6 mm column with L11 (Phenyl) packing as stationary phase, which is identical to conditions in the assay method also. No particle size mentioned wherefore the ratio must be calculated using the largest particle size consigned in the USP definition of the column.

System suitability requirements specify a chromatographic resolution not less than (NLT) 10 between fexofenadine and fexofenadine related compound A; a peak tailing factor of not more than (NMT) 2.0.

The following pages illustrate that the acceptance criteras are being met for Fexofenadine assay and its related substances methods by following the current USP monograph and using identical matched column. Using a 250x4.6 mm Purospher® STAR Phenyl (5 µm) column you can comply with the requirements for Fexofenadine assay and its related substances analysis.

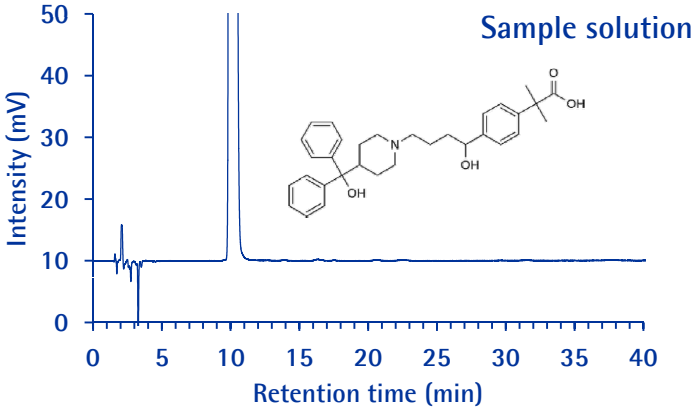
To address the current trend of monograph modernization, and improving the method in terms of selectivity, speed and sensitivity, we also scaled this method to a 100x2.1 mm UHPLC column; Purospher® STAR Phenyl (2 µm). The acceptance critera are met for the for Fexofenadine monograph. This is however, a non-allowed scaling and would require a complete revalidation of the new method for approval.

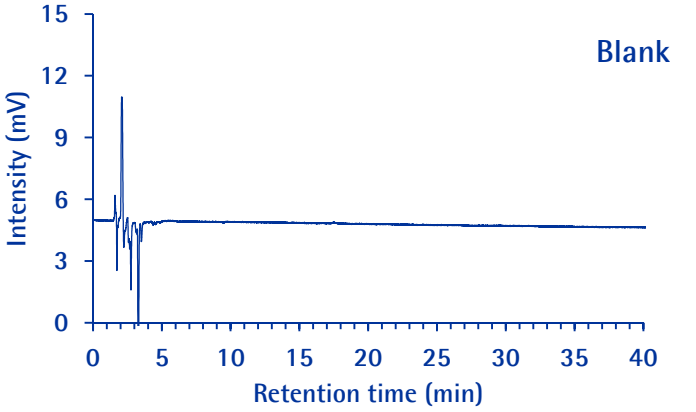
Fexofenadine and Related Substances (USP)

Purospher® STAR Phenyl – HPLC

Chromatographic Conditions

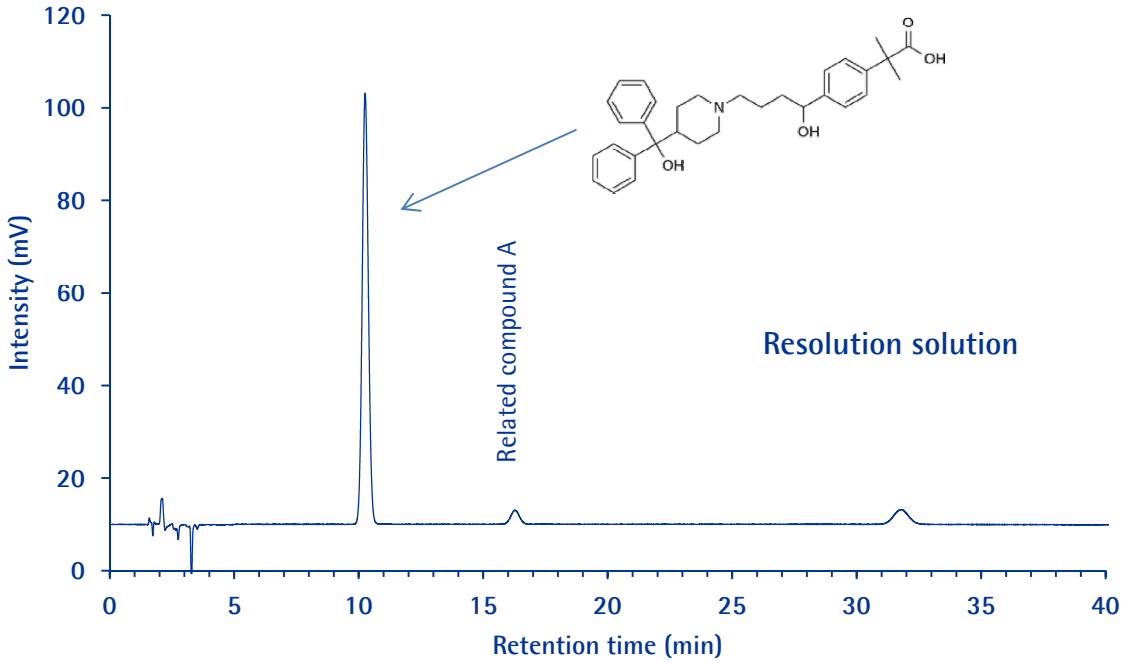
Column:	Purospher® STAR Phenyl (5µm) Hibar® RT 250x4.6 HPLC column	1.51918.0001
Injection:	20 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: 6.64 g/L of monobasic sodium phosphate and 0.84 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0. Mix acetonitrile and buffer 7:13 (v/v). Add 3 mL/L of triethylamine.	
Temperature:	25°C	
Diluent:	Acetonitrile and Buffer 1:1 (v/v)	
SST Solution:	0.06 mg/mL of Fexofenadine Hydrochloride RS and 0.005 mg/mL of Fexofenadine Related Compound A in Mobile phase	
Sample Solution:	1.0 mg/mL of Fexofenadine Hydrochloride in Diluent	
Pressure Drop:	164 Bar (2378psi)	





Fexofenadine and Related Substances (USP)

Purospher® STAR Phenyl – HPLC



Suitability requirement: NLT 10 in resolution
NMT 2.0 in Tailing factor

Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	Tailing Factor
1	Fexofenadine	10.3		1.1
2	Related compound A	16.3	10.9	1.0

Recommended chemicals and reagents

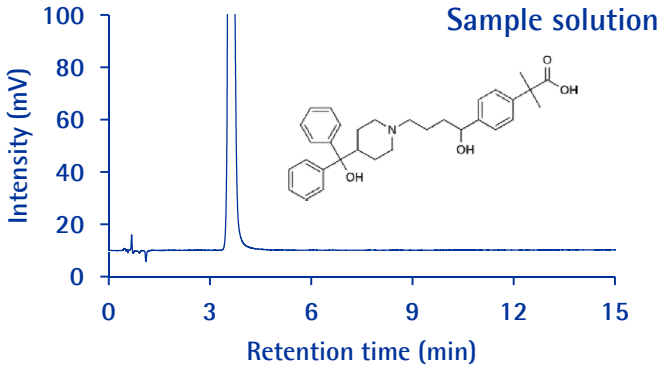
	Name of the chemical	Article Number
1	Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur	1.06342
2	Sodium perchlorate monohydrate for analysis EMSURE®	1.06564
3	ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00573
4	Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.00030

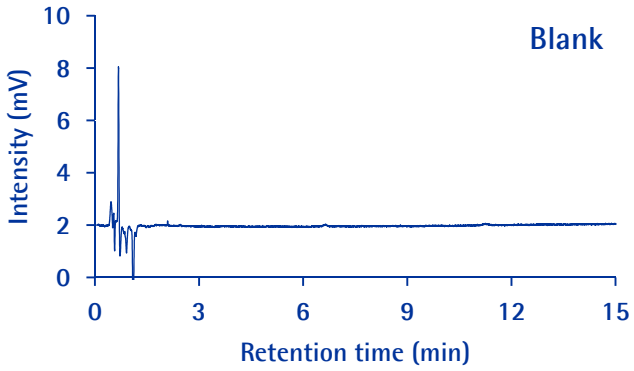
Fexofenadine and Related Substances (USP)

Purospher® STAR Phenyl – UHPLC

Chromatographic Conditions

Column:	Purospher® STAR Phenyl.(2 µm) Hibar® HR 100x2.1 mm	1.51014.0001
Injection:	2 µL	
Detection:	UV 220 nm	
Cell:	2.5 µL	
Flow Rate:	0.4 mL/min	
Mobile Phase:	6.64 g/L of monobasic sodium phosphate and 0.84 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.0. Mix Acetonitrile and buffer 7:13 (v/v). Add 3 mL/L of triethylamine.	
Temperature:	25°C	
Diluent:	Acetonitrile and Buffer 1:1 (v/v)	
SST Solution:	0.06 mg/mL of Fexofenadine Hydrochloride RS and 0.005 mg/mL of Fexofenadine Related Compound A in mobile phase	
Sample Solution:	1.0 mg/mL of Fexofenadine Hydrochloride in Diluent	
Pressure Drop:	388 Bar (5626psi)	





Scaling 250x4.6 to 100x2.1

Column volume reduction: 12X
(and thereby injection volume also)

Flow rate reduction: 12X
(to maintain same retention time)

Flow rate reduction: 4.8 X
(to maintain same linear velocity)

Injection volume should be:20/12= 1.67
but we used 2 µL (more practical)

Flow rate should either be 0.115 or 0.315 mL/min. **We used 0.4 mL/min to optimize analysis time and still comply with system suitability criterias**

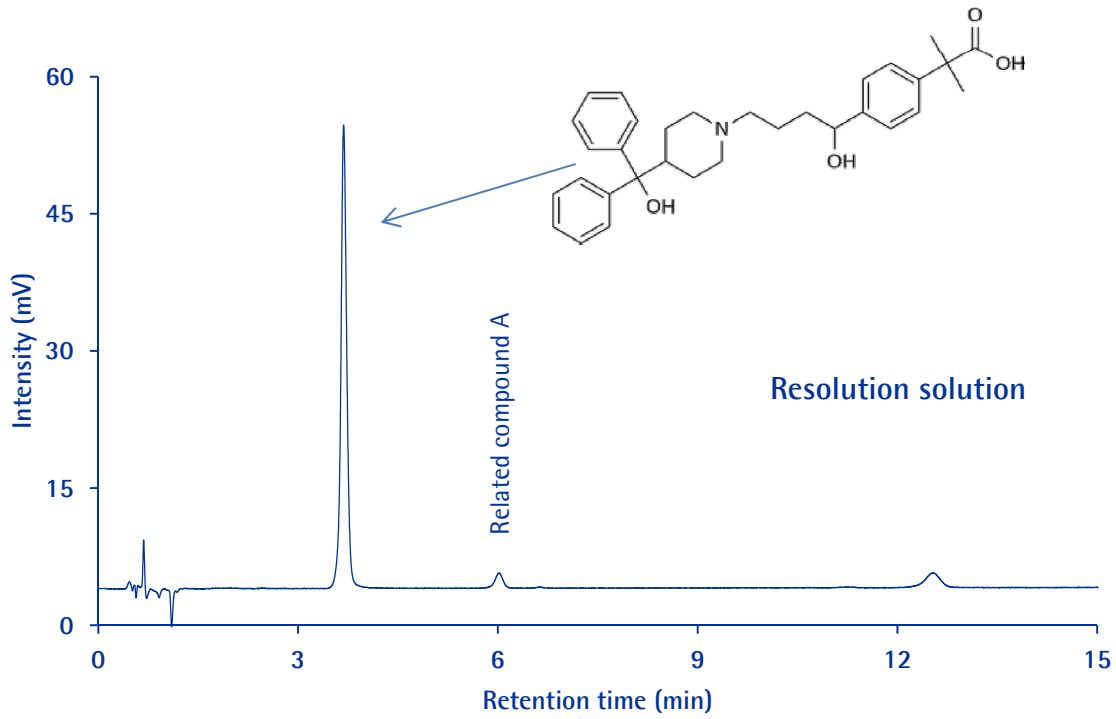
Flowrate per USP 37 change guidelines

$F2=1.5 \times (2.1^2 x5)/(4.6^{2'}2) = 0.78$ mL/min

This would be completely impractical and backpressure would be beyond tolerance of column and system.

Fexofenadine and Related Substances (USP)

Purospher® STAR Phenyl – UHPLC



Suitability requirement: NLT 10 in resolution
NMT 2.0 in Tailing factor

Chromatographic Data :

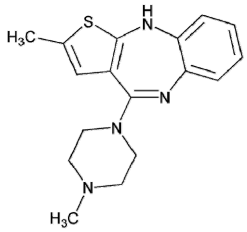
No.	Compound	Retention Time (min)	Resolution	Tailing Factor
1	Fexofenadine	3.7		1.0
2	Related compound A	6.0	12.7	1.0

Recommended chemicals and reagents

	Name of the chemical	Article Number
1	Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur	1.06342
2	Sodium perchlorate monohydrate for analysis EMSURE®	1.06564
3	ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00573
4	Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.00030

Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped



Olanzapine

Olanzapine is an atypical antipsychotic, approved for the treatment of schizophrenia and bipolar disorder. Common brand names: Zyprexa, Zalasta, Zolafren, Olzapin, Oferta, Zypadhera. Combination Drug: Symbyax (Olanzapine and Fluoxetine)

The current USP monograph method for Olanzapine and related substances specifies use of a 250x4.6 mm column with 5 µm L7 (RP-8) packing as stationary phase.
System suitability requirements for related substances are; the resolution between Olanzapine and Olanzapine related substance A should not be less than 3.0 and the tailing factor should not be more than 1.5 for the Olanzapine peak.

The following pages illustrate that the acceptance critera are being met for the Olanzapine and related substances method by following the current USP monograph using a 250x4.6 mm Purospher® STAR RP-8 endcapped (5 µm) column.

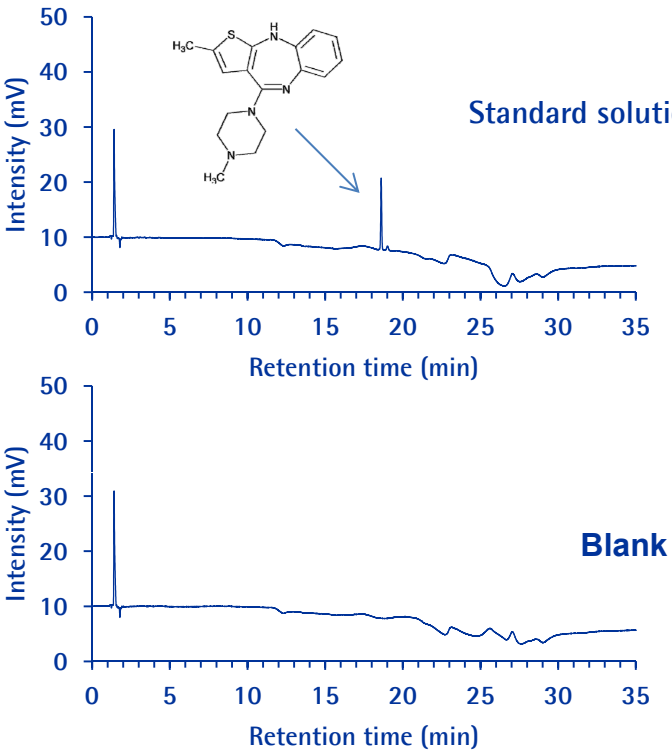
To address the current trend of monograph modernization, and improving the method in terms of selectivity, speed and sensitivity, we also scaled this method to a 100x2.1 mm UHPLC column; Purospher® STAR RP-8 endcapped (2 µm). Also in this example, all the system suitability critera are met for the Olanzapine and related substances. This is however, a non-allowed scaling and would require a complete revalidation of the new method for approval. For gradient separations, changes in length, column inner diameter and particle size are not allowed. This is new in USP 37.

Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped – HPLC

Chromatographic Conditions

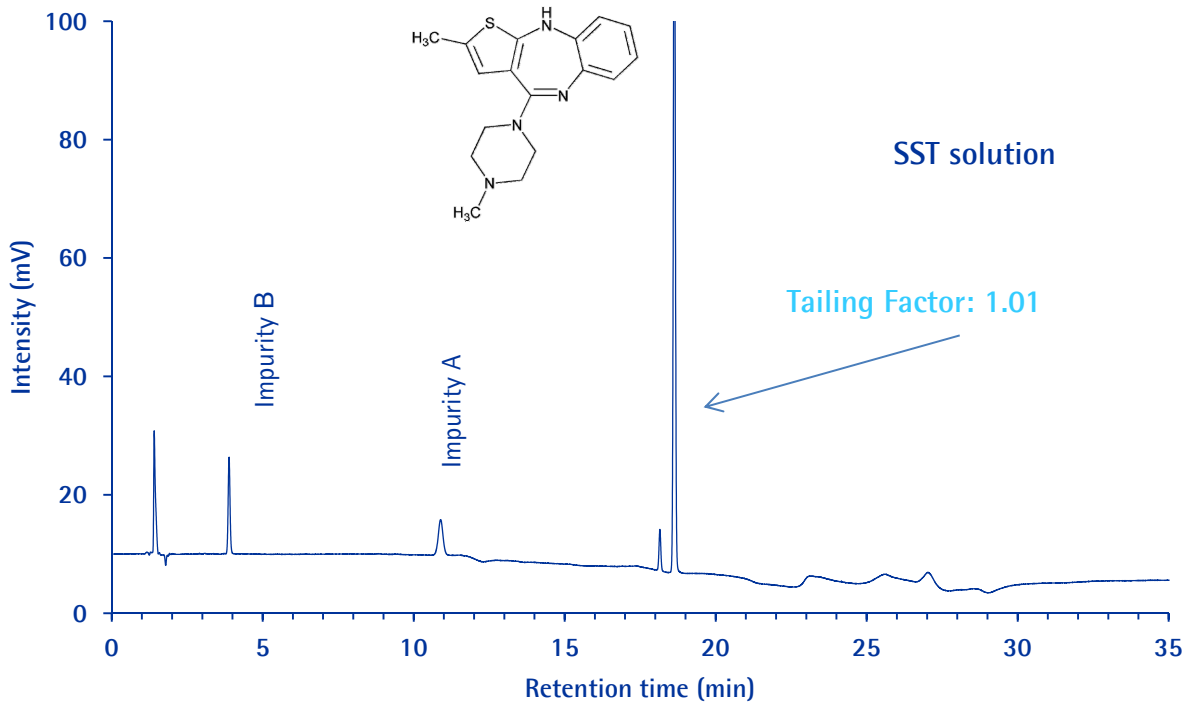
Column:	Purospher® STAR RP-8 endcapped (5µm) Hibar® 250x4.6 mm	1.51454.0001
Injection:	20 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water. Add 5 mL of phosphoric acid, and adjust with a sodium hydroxide solution to a pH of 2.5. A: Acetonitrile and Buffer 48:52 (v/v) B: Acetonitrile and Buffer 70:30 (v/v)	
Gradient:	See table	
Temperature:	35°C, Autosampler cooler at 5°C	
Diluent:	37 mg/L of EDTA disodium in Buffer. Acetonitrile and EDTA solution 40:60 (v/v)	
Test Solution:	20 µg/mL of USP Olanzapine, and 2 µg/mL each of Related Compound A and B in diluent	
Standard Solution:	2 µg/mL of USP Olanzapine in diluent	
Sample Solution:	0.4 mg/mL of Olanzapine in diluent	
Pressure Drop:	168 - 129 Bar (2434 - 1871 psi)	



Time (min)	A (%)	B (%)
0.0	100	0
8.0	100	0
17.0	70	30
20.0	70	30
20.1	100	0
23.0	100	0

Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped - HPLC



Chromatographic Data :

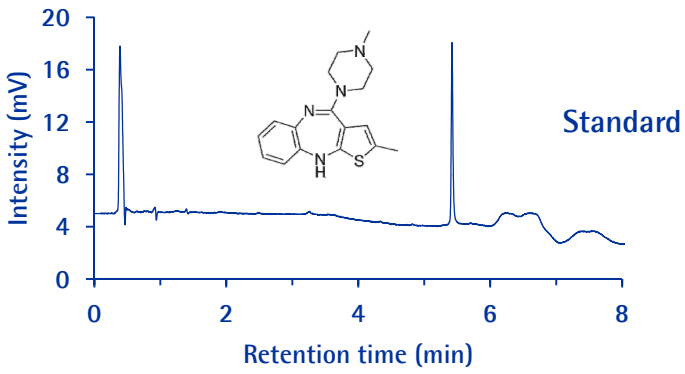
No.	Compound	Retention Time (min)	Resolution	RRT
1	Olanzapine Related compound B	3.9		0.21
2	Olanzapine Related compound A	10.9	34.3	0.59
3	Olanzapine	18.6	37.9	1.00

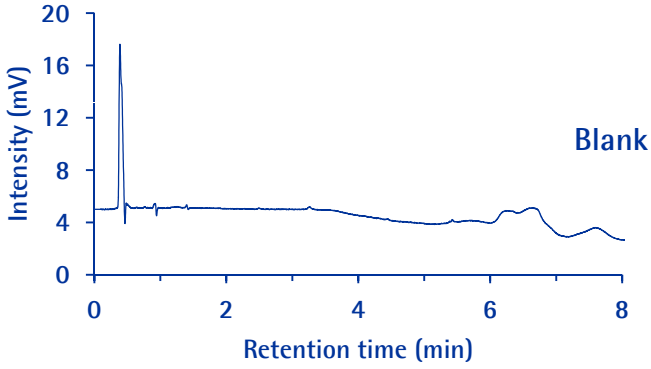
Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped – UHPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (2µm) Hibar® HR 100x2.1 mm	1.50629.0001
Injection:	5 µL *(larger injection volume than according to column tube volume reduction)*	
Detection:	UV 220 nm	
Cell:	2.5 µL	
Flow Rate:	0.5 mL/min *(faster flow rate – thus not according to same linear velocity)*	
Mobile Phase:	Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water. Add 5 mL of phosphoric acid, and adjust with a sodium hydroxide solution to a pH of 2.5. A: Acetonitrile and Buffer 48:52 (v/v) B: Acetonitrile and Buffer 70:30 (v/v)	
Gradient:	See table	
Temperature:	35°C, Sample cooler at 5°C	
Diluent:	37 mg/L of EDTA disodium in Buffer. Acetonitrile and EDTA solution 40:60 (v/v)	
Test Solution:	20 µg/mL of USP Olanzapine, and 2 µg/mL each of Related Compound A and Related Compound B in diluent	
Standard Solution:	2 µg/mL of USP Olanzapine in diluent	
Sample Solution:	0.4 mg/mL of Olanzapine in Diluent	
Pressure Drop:	408 - 311 Bar (5916 - 4510 psi)	





Time (min)	A (%)	B (%)
0.0	100	0
2.35	100	0
4.85	0	100
6.1	0	100
6.6	100	0
9.0	100	0

Scaling 250x4.6 to 100x2.1

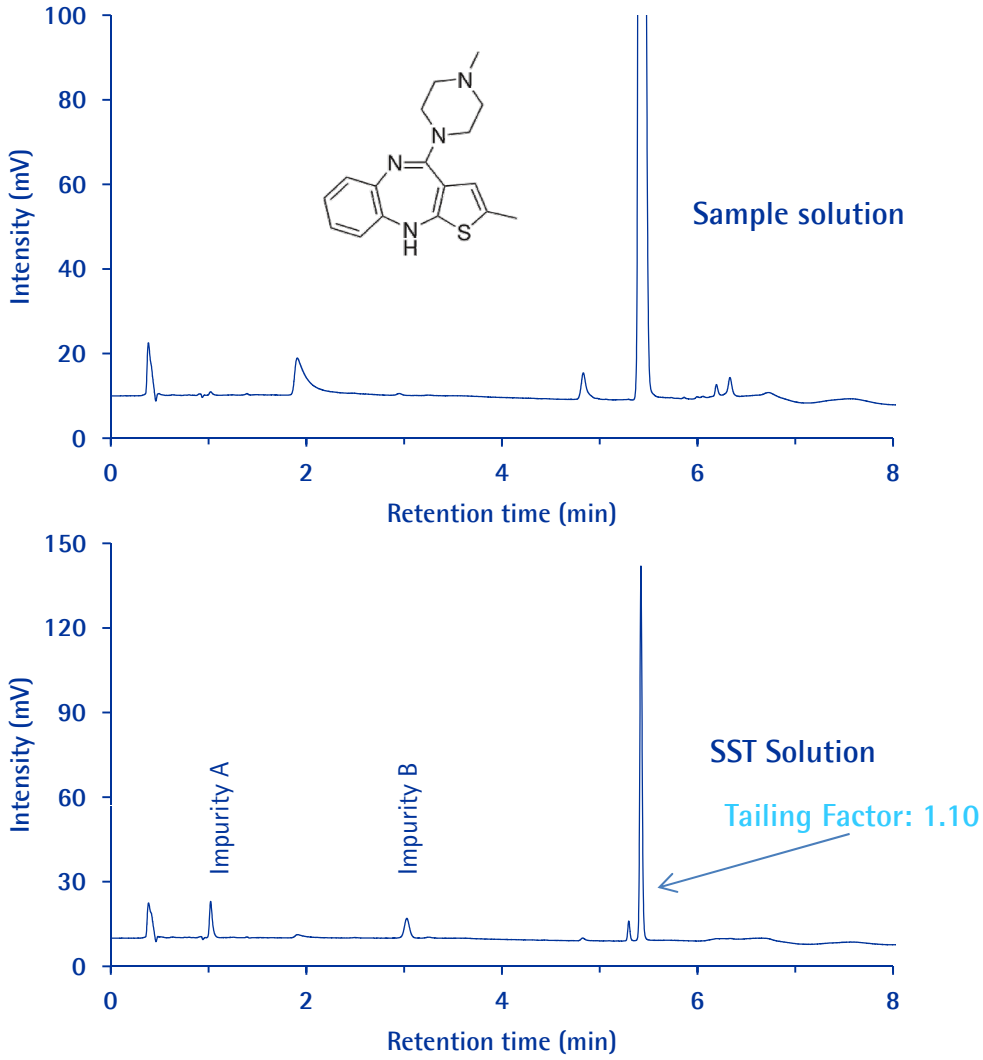
Column volume reduction: 12X
(and thereby injection volume also)

Flow rate reduction: 12X
(to maintain same retention time) or

Flow rate reduction: 4.8 X
(to maintain same linear velocity)

Olanzapine and Related Substances (USP)

Purospher® STAR RP-8 endcapped – UHPLC



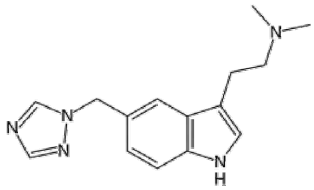
Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	RRT
1	Olanzapine Related compound A	1.0		0.19
2	Olanzapine Related compound B	3.0	26.1	0.55
3	Olanzapine	5.4	33.6	1.00

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

Rizatriptan and Related Substances (USP)

Purospher® STAR Phenyl



Rizatriptan

Rizatriptan is a 5-HT1 receptor agonist for the treatment of migraine headaches.
Common brand name is Maxalt.

The current USP monograph method for Rizatriptan and related substances as well as the assay method specifies the use of a 250x4.6 mm column with 5 µm L11 (Phenyl) packing as stationary phase. System suitability criteria in the assay method specify that the relative retention times for rizatriptan and benzoic acid are 1.0 and about 2.1, respectively., as well as a tailing factor NMT 3.5 for rizatriptan. System suitability requirements for related substances are; the relative retention times for rizatriptan, rizatriptan impurity C, and benzoic acid are 1.0, about 1.3, and about 2.1 RRT. The resolution is NLT 2.0 between rizatriptan and rizatriptan impurity C; and using the sensitivity solution the signal-to-noise ratio should be NLT 10 for the rizatriptain peak.

The following pages illustrate that the acceptance criteras are met for the assay and there is higher resolution and retention for the impurites in the Rizatriptan and related substances method by following the current USP monograph using a 250x4.6 mm Purospher® STAR Phenyl (5 µm) column.

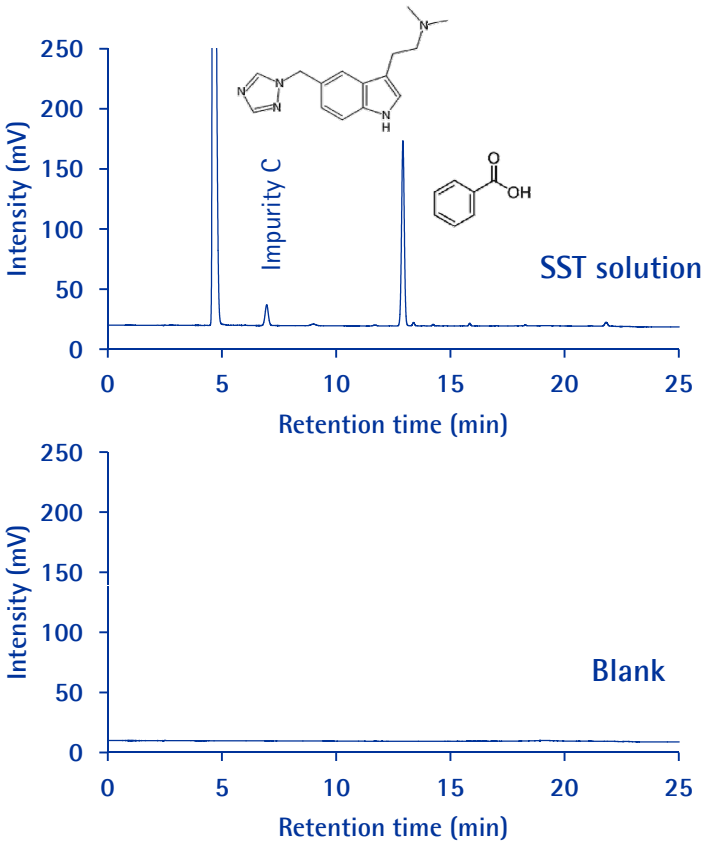
To address the current trend of monograph modernization, and improving the method in terms of selectivity, speed and sensitivity, we also scaled this method to a 100x2.1 mm UHPLC column; Purospher® STAR Phenyl (2 µm). Also in this example, all the system suitability critera are met. This is however, a non-allowed scaling and would require a complete revalidation of the new method for approval. For gradient separations, changes in length, column inner diameter and particle size are not allowed. This is new in USP 37.

Rizatriptan and Related Substances (USP)

Purospher® STAR Phenyl – HPLC

Chromatographic Conditions

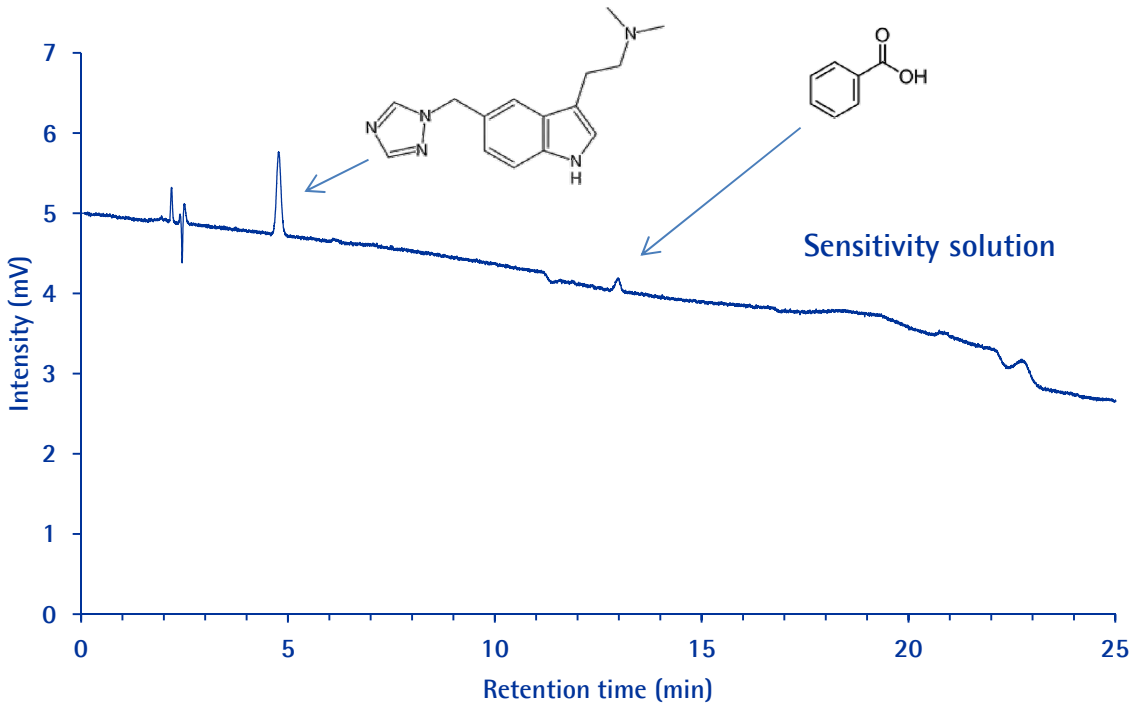
Column:	Purospher® STAR Phenyl (5µm) Hibar® RT 250x4.6 mm	1.51918.0001
Injection:	20 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	A: 1.0 mL of trifluroacetic acid to 1 L of a solution of acetonitrile and Water 4:21 (v/v), and mix. B: Acetonitrile and trifluroacetic acid 1000:1 (v/v) (or use our premixed product 4.80448)	
Gradient:	See table	
Temperature:	40°C	
Diluent:	Solution A	
Test Solution:	1 mg/mL of USP Rizatriptan Benzoate System Suitability Mixture in Solution A	
Resolution Solution:	0.5 µg/mL of Rizatriptan Benzoate obtained by suitable dilution of the Sample solution with Solution A	
Pressure Drop:	140 - 129 Bar (2030 - 1871 psi)	



Time (min)	A (%)	B (%)
0.0	100	0
8.0	100	0
17.0	70	30
20.0	70	30
20.1	100	0
23.0	100	0

Rizatriptan and Related Substances (USP)

Purospher® STAR Phenyl – HPLC



Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	RRT
1	Rizatriptan	4.6		1.00
2	Impurity C	7.0	9.1	1.52
3	Benzoic acid	12.9		2.80

	USP Specification	Observed value
S/N ratio of Sensitivity solution	NLT 10	12.6

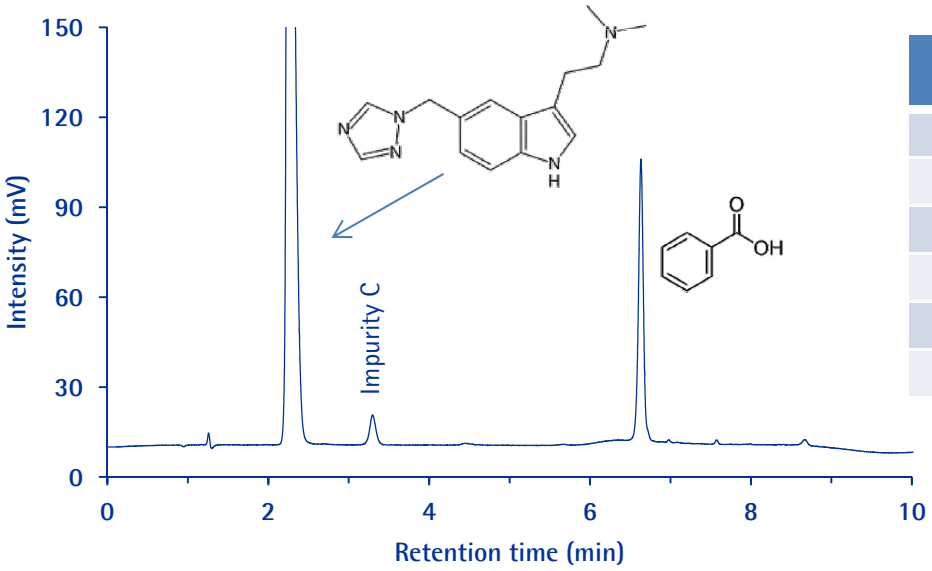
The benzoic acid retention time is somewhat larger than the specified value in the current monograph (and more than ± 10% of tabulated RRT). This deviation would most likely be approved benzoic acid is both well separated from the API and not interfering with the analysis of any other impurities.

Rizatriptan and Related Substances (USP)

Purospher® STAR Phenyl – UHPLC

Chromatographic Conditions

Column:	Purospher® STAR Phenyl (2µm) Hibar® HR 100x2.1 mm	1.51014.0001
Injection:	2 µL *(appropriate scaling per column tube volume reduction)*	
Detection:	UV 280 nm	
Cell:	2.5 µL	
Flow Rate:	0.25 mL/min *(not scaled according to linear velocity)*	
Mobile Phase:	A: Add 1.0 mL of trifluroacetic acid to 1 L of a solution of acetonitrile and water; 4:21 (v/v) B: Acetonitrile and trifluroacetic acid 1000:1 (v/v) (or use our premixed product 4.80448)	
Gradient:	See table	
Temperature:	40°C	
Diluent:	Solution A	
Test Solution:	1 mg/mL of USP Rizatriptan Benzoate System Suitability Mixture in Solution A	
Resolution Solution:	0.5 µg/mL of Rizatriptan Benzoate obtained by suitable dilution of the Sample solution with Solution A	
Pressure Drop:	205 - 195 Bar (2973 - 2828 psi)	



Time (min)	A (%)	B (%)
0.0	100	0
2.55	100	0
5.67	70	30
6.67	70	30
6.7	100	0
10.0	100	0

Chromatographic Data :

No.	Compound	Retention Time (min)	Resolution	RRT
1	Rizatriptan	2.2		1.00
2	Impurity C	3.3	6.7	1.50
3	Benzoic acid	6.6		3.00

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
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chromatography@merckgroup.com

Solvents and Reagents

Product	P/N
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00063
Acetonitrile isocratic grade for liquid chromatography LiChrosolv®	1.14291
Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.00030
Ammonia solution 28-30% for analysis EMSURE® ACS,Reag. Ph Eur	1.05423
Ammonium acetate for analysis EMSURE® ACS,Reag. Ph Eur	1.01116
Ammonium dihydrogen phosphate for analysis EMSURE® ACS,Reag. Ph Eur	1.01126
N,N-Dimethylformamide for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.03053
Ethanol gradient grade for liquid chromatography LiChrosolv®	1.11727
Hydrochloric acid fuming 37% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00317
Methanol for liquid chromatography LiChrosolv®	1.06018
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.06007
Octane-1-sulfonic acid sodium salt for ion pair chromatography LiChropur®	1.18307
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00573
Perchloric acid 70% for analysis (max. 0.0000005% Hg) EMSURE® ACS,ISO,Reag. Ph Eur	1.00514
Potassium dihydrogen phosphate anhydrous 99.995 Suprapur®	1.05108
di-Potassium hydrogen phosphate trihydrate buffer substance for chromatography	1.19754
Potassium hydroxide pellets for analysis EMSURE®	1.05033
Sodium acetate anhydrous 99.99 Suprapur®	1.06264
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur	1.06342
di-Sodium hydrogen phosphate dihydrate for analysis EMSURE®	1.06580
Sodium hydroxide pellets for analysis (max. 0.0002% K) EMSURE® ACS,Reag. Ph Eur	1.06495
Sodium perchlorate monohydrate EMSURE®	1.06564
Tetrahydrofuran LiChrosolv®	1.08101
Acetonitrile with 0.1% (v:v) trifluoroacetic acid for liquid chromatography LiChrosolv®	4.80448
Water for chromatography*	1.15333
* or use a Milli-Q Integral Water Purification System	

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