Complete
Monograph Methods

Solutions for regulated Pharmaceutical Instrumental Analysis
Methods with HPLC, FTIR, KF, AAS and ICP

2015 –1

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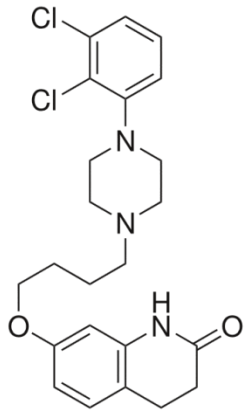
Molecular Structures



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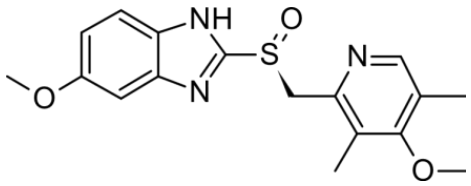
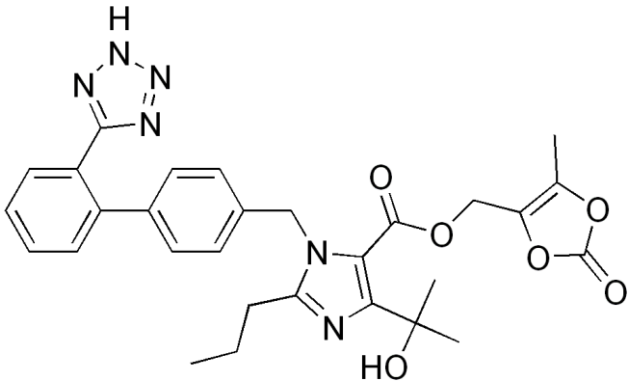
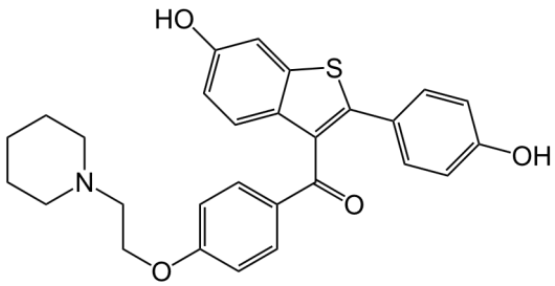


Esomeprazole



Olmesartan medoxomil



Raloxifene

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Introduction

This compilation presents complete solutions for regulated pharmaceutical instrumental analysis with focus on the testing of both generic as well as ethical small molecule drugs. All highlighted methods (18 in total) follow their current USP37-NF32 monographs, and all are compliant with requirements in the upcoming USP 38-NF33.

A monograph represents published standard methods by which the use of one or more substances is authorized. By following the specific method(s) and complying with specifications a manufacturer can prove the safety of their products; yet this does not mean it will automatically be approved.

USP–NF is a combination of the United States Pharmacopeia (USP) and the National Formulary (NF). Monographs for drug substances, dosage forms, and compounded preparations are shown in the USP; monographs for dietary supplements and ingredients can be found in a separate section of the USP, and monographs for excipients can be found in the NF.

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

In this compilation you will find HPLC and UHPLC methods for active pharmaceutical ingredient (API) assays; their related substances (impurity profiling) as well as dissolution testing of a formulated drug (Esomeprazole delayed release capsules). You will also find Karl Fischer (KF) methods for water determination, Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma (ICP) methods for metal content determination, and FTIR analysis for identification purposes. We have even developed a new LC-MS procedure for impurity profiling of Olmesartan medoxomil, but this is not an active USP method. All methods are compliant with the system suitability criteria of each corresponding monograph.

In all examples, we have used high quality products from Merck Millipore which easily can be used for your needs in validated pharmaceutical quality control.

USP General Chapters

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

In the general chapters you can find details about different tests and procedures referred to in multiple monographs, and in the general notices definitions for terms used in the monographs, as well as information that is necessary to interpret the monograph requirements are found.
The following pages show some of these details that are relevant for the analytical techniques used within this compilation, namely chapter 197 (relevant for identification with IR or FTIR), 232-233 (relevant for heavy metal analysis with AAS or ICP), 621 (chromatography), 711 (dissolution) and 921 (water determination/Karl Fischer titration).

Reference Standards

"Reference Standards provided by the USP 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

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.

Chapter 197 – IR

Spectrophotometric tests are used for **identification** of many compendial chemical substances. The test procedures that follow are applicable to substances that absorb IR and/or UV radiation (Chapter 851 - Spectrophotometry and Light-Scattering). The IR absorption spectrum of a substance, compared with that obtained for the corresponding USP Reference Standard, provides perhaps the most conclusive evidence of the identity of the substance that can be realized from any single test. Seven methods are indicated for the preparation of previously dried specimens and Reference Standards for analysis:

-197K signifies that the substance is mixed intimately with potassium bromide.

-197M signifies that the substance is finely ground and dispersed in mineral oil.

-197F signifies that the substance is suspended neat between suitable (for example, sodium chloride or potassium bromide) plates.

-197S signifies that a solution of designated concentration is prepared in the solvent specified in the individual monograph, and the solution is examined in 0.1-mm cells unless a different cell path length is specified in the individual monograph.

- 197A signifies that the substance is intimately in contact with an internal reflection element for attenuated total reflectance (ATR) analysis.

-197E signifies that the substance is pressed as a thin sample against a suitable plate for IR microscopic analysis.

-197D in a monograph signifies that the substance is mixed intimately with an IR-transparent material and transferred to a sample container for diffuse reflection (DR) analysis. The ATR 197A and the 197E techniques can be used as alternative methods for 197K, 197M, 197F, and 197S where testing is performed qualitatively and the Reference Standard spectra are similarly obtained.

How to proceed?
Record the spectra of the test specimen and the corresponding USP Reference Standard over the range from about 2.6 µm to 15 µm (3800 cm−1 to 650 cm−1) unless otherwise specified in the individual monograph. The IR absorption spectrum of the preparation of the test specimen, previously dried under conditions specified for the corresponding Reference Standard unless otherwise specified, or unless the Reference Standard is to be used without drying, exhibits maxima only at the same wavelengths as that of a similar preparation of the corresponding USP Reference Standard.

USP chapter 232 and 233 – ICP

For more than a hundred years heavy metal impurity analysis has been a common requirement in many monographs. Currently this test is regulated under general chapter 231, and it demonstrates that the content of metallic impurities, colored by sulfide ion, does not exceed the heavy metals limit specified in the individual monograph of lead in the test substance. It is a visual comparison with a control prepared from a standard lead solution, and therefore a qualitative test.

We have not carried out this test for any of the Aripriprazole, Olmesartan medoxomil or Raloxifene monographs, beacuse chapter 231 will, in a near future, be replaced with the two new general chapters 232 and 233. USP 232 "Elemental Impurities – Limits" and 233 "Elemental Impurities– Procedure" propose that the testing for heavy metals in pharmaceutical products can be performed by means of ICP-OES and ICP-MS (Inductively Coupled Plasma Optical Emission Spectrometry and ICP-Mass Spectrometry). These new chapters became official February 1, 2013 in the Second Supplement to USP 35–NF 30.

It is important to note that revisions to General Chapters <232> and <233> were proposed in Pharmacopeial Forum 40(2) [March-April 2014] with changes related to the ICH Q3D Step 2 document as well as other editorial changes. General Chapters <232> and <233> will thus remain official in their current form until the revisions published in PF 40(2) become official. The new deadline of introduction of the USP <232> has been postponed to 2018. This means that the USP <231> is still active, yet this will be omitted once General Chapters <232> and <2232> become applicable. This means all stakeholders will have until 2018 to change their analytical methodology for carrying out the determination of elemental impurities to be consistent with the limits and procedures described in these two new chapters.

Advantages of ICP-based analyses include considerable reductions in sample intake (mg instead of gram quantities) and the ability to generate quantitative results. Chapter USP 232 lists two different classes of elements; Class 1 – As, Cd, Hg, and Pb, that are obligatory for all drug products; and Class 2 – elements that need to be monitored if added or used in the production process. USP 233 includes information about two reference methods, one with ICP-OES and the other ICP-MS, as well as instructions for the validation of both limit test and quantitative procedures.

To exemplify this change we have tested Olmesartan medoxomil RS with ICP-MS, and you can find a suggested procedure in this compilation.

USP Chapter 621 – Chromatography

What changes are allowed in a monograph method?

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

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

Factors that may affect chromatographic behavior:

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

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

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

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

In some circumstances, it may be desirable to use an HPLC column with different dimensions to those prescribed in the official procedure (different length, internal diameter, and/or particle size). 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.

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

USP Packings (L classifications)
Merck Millipore Columns

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

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

	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.

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

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

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

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

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

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

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

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

Changes in USP37

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

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

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

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

Ratio of Components in Mobile Phase

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

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

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

Wavelength of UV-Visible Detector

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

Choosing the right Column to meet Monograph Specifications

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

The USP also has a database for chromatography columns to help users cross-reference HPLC columns. 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, 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."*

We at Merck Millipore are confident that our columns can meet monograph specifications despite the fact that they may seem very different from the column used when developing the original monograph method. 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.

Validation and Verification

The process of validating a new analytical procedure for compendial usage is addressed in USP general Chapter 1225 – "Validation of Compendial Procedures". However, even with a fully validated procedure, the end-user may not have assurance that the procedure is suitable for use with a specific ingredient or product in a specific laboratory with specific personnel, equipment, consumables and reagents. USP therefore developed chapter 1226 in response to industry's request to provide instructions for verifying compendial procedures in specific situations.
Here we have addressed USP's proposed new general chapter 1226 "Verification of Compendial Procedures" which is intended to fill the gap in the proper usage of compendial procedures by outlining a process for verifying their suitability. The role of HPLC columns is of immense importance to meet system suitability test (SST) criteria in compendial methods.

Validation of Compendial Procedure <1225>

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.

USP Chapter 711 – Dissolution

Dissolution testing is used to determine compliance where stated in individual monographs for dosage forms administered orally. The text in the general chapter 711 is harmonized with the European Pharmacopeia and/or the Japanese Pharmacopeia. These three pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter.

A dosage unit is defined as 1 tablet or 1 capsule or the amount specified.
The type of instrument used should follow the specification in the individual monograph.

Where the label states that it is enteric-coated, and where a dissolution or disintegration test that does not specifically state that it is to be applied to delayed-release articles is included in the individual monograph, the procedure and interpretation given for Delayed-Release Dosage Forms is applied unless otherwise specified in the individual monograph.

For hard or soft gelatin capsules and gelatin-coated tablets that do not conform to the Dissolution specification, repeat the test as follows. Where water or a medium with a pH of less than 6.8 is specified as the Medium in the individual monograph, the same Medium specified may be used with the addition of purified pepsin that results in an activity of 750,000 Units or less per 1000 mL. For media with a pH of 6.8 or greater, pancreatin can be added to produce not more than 1750 USP Units of protease activity per 1000 mL.

In this compilation we have tested **Esomeprazole delayed release capsules** using the following medium: 0.1 N hydrochloric acid; 300 mL. After 2 h, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 mL of 0.086 M dibasic sodium phosphate, and adjust with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05.

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

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

More details can be found by studying the data in the Esomeprazole Delayed Release monograph herein.

USP Chapter 921 Water Determination

Many Pharmacopeial articles either are hydrates or contain water in adsorbed form.
The determination of the water content is therefore important in demonstrating compliance with the Pharmacopeial standards. When the article contains water of hydration, Method I (Titrimetric), Method II (Azeotropic), or Method III (Gravimetric) is employed.

The United States Pharmacopeia (USP) has three compendial methods for water determination **921-1a (Direct Titration), 1b (Residual Titration) and 1c (Coulometric Titration)**, whereas the European Union uses mostly method EP (2.5.12), which is equivalent to USP chapter 921-1c; determination of content uniformity in JP General Test 109, even if it is considered an equivalent method to USP chapter 905, has differences in the way the results are calculated and reported.

In scope of the USP chapter 921, we have included one example in this compilation illustrating how to perform volumetric Karl Fischer (KF) water determination (1a) – Esomeprazole; and one example with coulometric Karl Fischer (KF) water determination (1c) – Olmesartan medoxomil.

The working principle of Method 1a is titrimetric determination of water using a quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions. In the original titrimetric solution, known as Karl Fischer Reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The test specimen may be titrated with the Reagent directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of this reaction is not exact, and the results depend on the relative concentrations of the reagents, the nature of the inert solvent used to dissolve the test sample, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy.

The working principle of Method 1c is that the Karl Fischer reaction is used in the coulometric determination of water. Iodine is not added in the form of a volumetric solution but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may also be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which usually is detected electrometrically, thus indicating the endpoint. Moisture is eliminated from the system by pre-electrolysis. Changing the Karl Fischer solution after each determination is not necessary because individual determinations can be carried out in succession in the same reagent solution.

USP Chapter 921 Water Determination

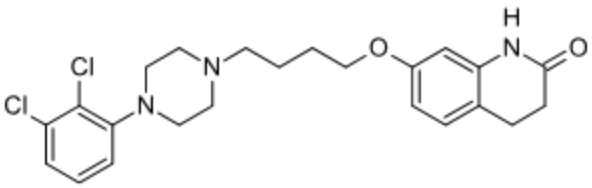
Reagents for Method Ia (prepare the Karl Fischer Reagent as follows**)**
Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250-mL graduated cylinder, and, keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One mL of this solution when freshly prepared is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 h before use, or daily if in continuous use. Protect from light while in use. Store any bulk stock of the reagent in a suitably sealed, glass-stoppered container, fully protected from light, and under refrigeration. For determination of trace amounts of water (less than 1%), it is preferable to use a Reagent with a water equivalency factor of not more than 2.0, which will lead to the consumption of a more significant volume of titrant. A commercially available, stabilized solution of Karl Fischer type reagent may be used.

Test Preparation: Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 2–250 mg of water.

Standardization of the Reagent: Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient Reagent to give the characteristic endpoint color, or 100 ± 50 microamperes of direct current at about 200 mV of applied potential.

Reagents for Method Ic – it says see the manufacturer's recommendations. Therefore we have included a method with recommended procedure to proceed with the analysis of Olmesartan medoxomil. If you need guidance or suggestions with appropriate reagents just send an email to: apura@merckgroup.com

Aripiprazole (USP)



Aripiprazole is an atypical antipsychotic, and it is a partial dopamine agonist.
It is primarily used in the treatment of schizophrenia, bipolar disorder, major depressive disorder, tic disorders, and irritability associated with autism.

First approved by the U.S. Food and Drug Administration (FDA) for schizophrenia in November 2002 and the European Medicines Agency in June 2004; for acute manic and mixed episodes associated with bipolar disorder.

Common commercial brand names: Abilify and Aripiprex

Aripiprazole was developed by Otsuka in Japan, and in the United States, Otsuka America markets it jointly with Bristol-Myers Squibb.

Sales in 2010 were $4.6 billion globally.
Patent expires in 2015

In this compilation, we have followed the methods for:
Identification – FTIR (197K)
Assay – HPLC (gradient method)
Related Substances - HPLC (gradient method)

The HPLC methods are gradient methods, thus non-scalable.
The same chromatographic conditions are used for both assay and related substances methods, and a full validation protocol can be found using USP reference standards; USP Aripiprazole RS and USP Aripiprazole Related Compound F RS.

Aripiprazole (USP)

Definition:

Aripiprazole contains NLT (not less than) 98.0% and NMT (not more than) 102.0% of Aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$), calculated on the dried basis.

Identification:

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

-B. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay. .

Assay: HPLC

-Procedure: (Protect the solutions from light.)
Diluent: Acetonitrile, methanol, water, and acetic acid (30:10:60:1)
Solution A: Acetonitrile and 0.05% trifluoroacetic acid (10:90)
Solution B: Acetonitrile and 0.05% trifluoroacetic acid (90:10)

Gradient: See Table.

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

[Note—The gradient was established on an HPLC system with a dwell volume of approximately 650 µL.
Chromatographic system: (See Chromatography 621, System Suitability.)

Detector: UV 254 nm
Column: 4.6-mm × 10-cm; 3 µm packing L1
Flow rate: 1.2 mL/min
Injection volume: 20 µL

We have used a Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm (1.50469.0001).
This is a gradient method and can therefore not be changed.

Aripiprazole (USP)

System suitability solution: 1 µg/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound F RS in Diluent
Standard solution: 0.1 mg/mL of USP Aripiprazole RS in Diluent
Sample solution: 0.1 mg/mL of Aripiprazole in Diluent

System suitability
Samples: System suitability solution and Standard solution
The relative retention times (RRT) for aripiprazole and aripiprazole related compound F are 1.0 and 1.1, respectively.

Suitability requirements
Resolution: NLT 2.0 between aripiprazole and aripiprazole related compound F, System suitability solution
Tailing factor: NMT 1.5 for aripiprazole, System suitability solution
Relative standard deviation: NMT 1.0%, Standard solution

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$) in the portion of the sample taken:

Result = (rU/rS) × (CS/CU) × 100

rU = peak area from the Sample solution
rS = peak area from the Standard solution
CS = concentration of USP Aripiprazole RS in the Standard solution (mg/mL)
CU = concentration of Aripiprazole in the Sample solution (mg/mL)

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

IMPURITIES
A. Residue on Ignition 281: NMT 0.1%
B. Heavy Metals, Method II 231: NMT 10 ppm

Organic Impurities (Protect the solutions from light.)
Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Aripiprazole (USP)

Analysis
Sample: Sample solution
Calculate the percentage of each impurity in the portion of Aripiprazole taken:

Result = (ri/rU) × (1/F) × 100

ri = peak response of each impurity from the Sample solution
rU = peak response of aripiprazole from the Sample solution
F = relative response factor (see Table 2)

Name	RRT	RRF	Acceptance criteria (NMT (%))
Aripiprazole related compound G[a]	0,9	0.72	0.10
Aripiprazole	1.0	-	-
Aripiprazole related compound F[b,c]	1.1	1.0	-
Aripiprazole 4,4-dimer[d]	1.3	1.0	0.10
Any other impurity	-	-	0.10
Total Impurities	-	-	0.50

a) 7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}quinolin-2(1H)-one.
b) 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazin 1-oxide.
c) For system suitability and identification purposes only.
d) 1,1¢-(Ethane-1,1-diyl)bis(2,3-dichloro-4-{4-[3,4-dihydroquinolin-2(1H)-one-7-yloxybutyl]piperazin-1-yl}benzene).

USP Reference Standards
USP Aripiprazole RS
USP Aripiprazole Related Compound F RS

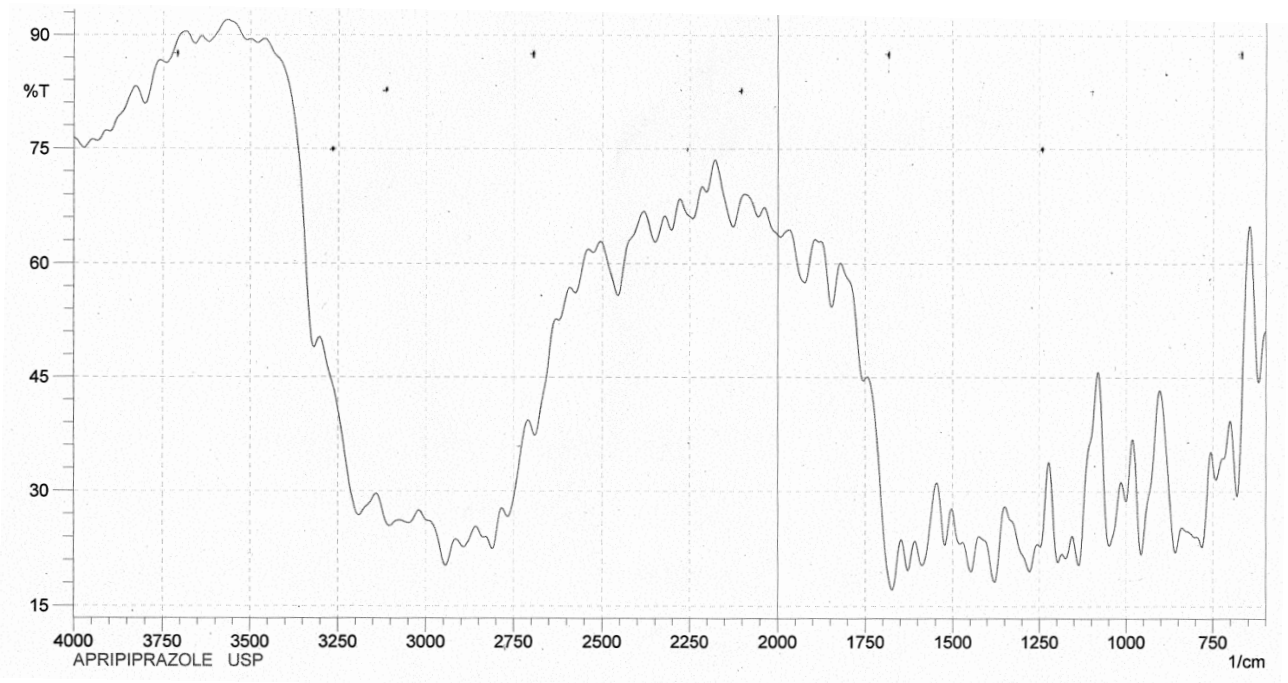
Recommended Merck Millipore products:
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur 1.00063
Acetonitrile (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur 1.00030
Methanol (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur 1.06007
Potassium bromide for for IR spectroscopy Uvasol® (1.04907)
Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm 1.50469
Trifluoroacetic acid for spectroscopy Uvasol® 1.08262
Water for chromatography (LC-MS Grade) LiChrosolv® 1.15333 or fresh water from Milli-Q system.

Aripiprazole Assay and Related Substances

Identification

A. INFRARED ABSORPTION <197K> FTIR

The reference 197K in a monograph signifies that the substance under examination is mixed intimately with potassium bromide. We recommend potassium bromide for IR spectroscopy Uvasol® (1.04907).

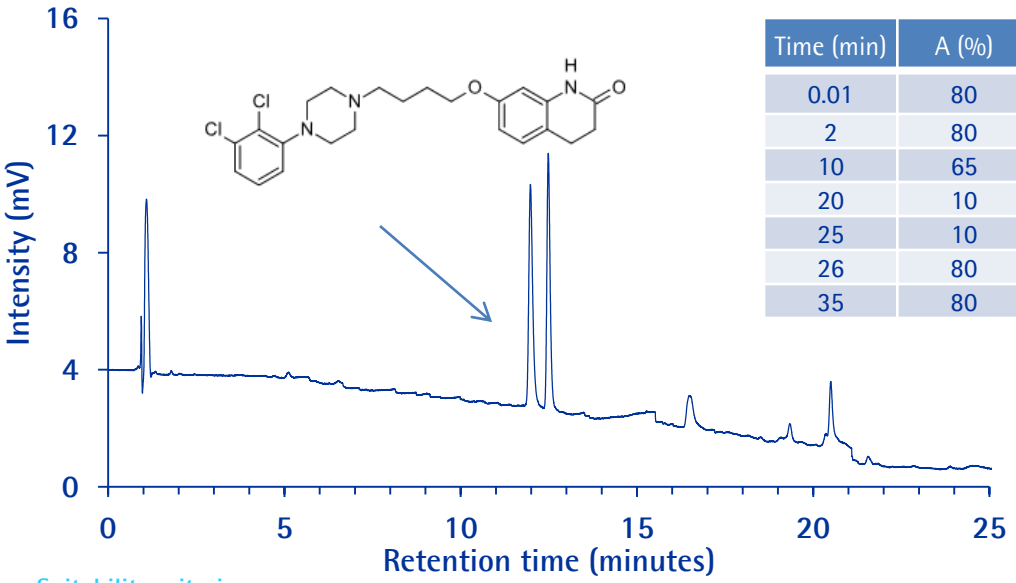


Aripiprazole Assay and Related Substances

Purospher STAR® RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm	1.50469.0001
Injection:	20 µL	
Detection:	UV 254 nm	
Cell:	10 µL	
Flow Rate:	1.2 mL/min	
Mobile Phase A:	Acetonitrile and 0.05% trifluoroacetic acid (10:90 v/v)	
Mobile Phase B:	Acetonitrile and 0.05% trifluoroacetic acid (90:10 v/v)	
Gradient:	See table	
Temperature:	40°C	
Diluent:	Acetonitrile:Methanol:Water:Acetic acid (30:10:60:1 v/v)	
Sample:	1 µg/mL (1ppm) each of Aripiprazole and Aripiprazole Related Compound F in diluent	
Pressure Drop:	95-170 Bar (1377-2465 psi)	



Time (min)	A (%)	B (%)
0.01	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

System Suitability criteria:
Resolution: NLT 2.0 between aripiprazole and aripiprazole related compound F
Tailing factor: NMT 1.5 for aripiprazole
RRT is 1.0 and 1.1 for aripiprazole and aripiprazole related compound F, respectively

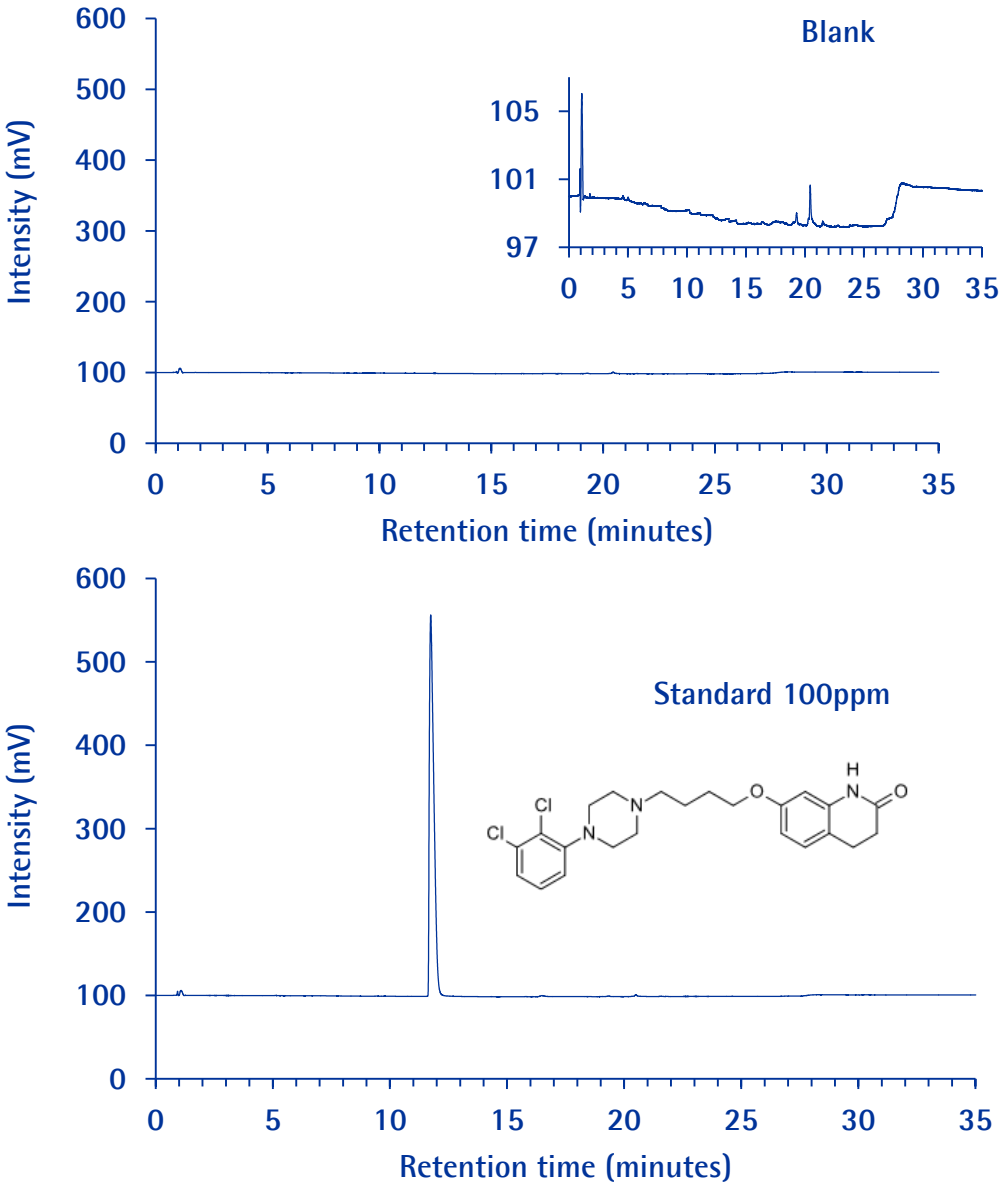
Chromatographic Data: SST solution

No.	Compound	Retention Time (min)	RRT	Tailing factor	Resolution
1	Aripiprazole	12.0	1.0	1.4	-
2	Aripiprazole RS F	12.5	1.05	1.3	2.8

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

Aripiprazole Assay and Related Substances

Purospher STAR® RP-18 endcapped



Chromatographic Data: Standard 100ppm

No.	Compound	Retention Time (min)	Tailing factor	Theoretical plates
1	Aripiprazole	12.0	1.4	16118

Aripiprazole Assay and Related Substances

Validation and Verification Data

1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Aripiprazole RS and Aripiprazole Related Compound F

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Aripiprazole	12.0	-	1.4	-
Aripiprazole RS F	12.5	1.05	1.3	2.8

2. **Repeatability.**
   Determined by injecting five (5) samples with a solution containing 100 ppm Aripiprazole and 1 ppm of Aripiprazole Related Compound F

	Aripiprazole (Area units)	Aripiprazole RS F (Area units)
Standard 1	60114	6047450
Standard 2	60363	6080108
Standard 3	60308	6086256
Standard 4	60174	6081386
Standard 5	60316	6089267
Mean	60255	6076893
Stdev	105.6	16868.6
RSD (%)	0.18	0.28

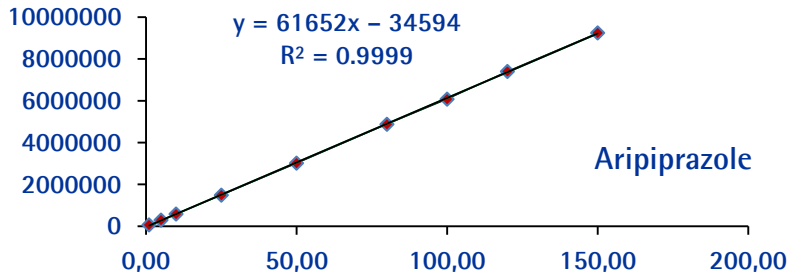
3. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (7) concentration levels from 0.1-1.5 ppm of Aripiprazole Related Compound F, and nine (9) concentration levels ranging from 1-150 ppm of Aripiprazole

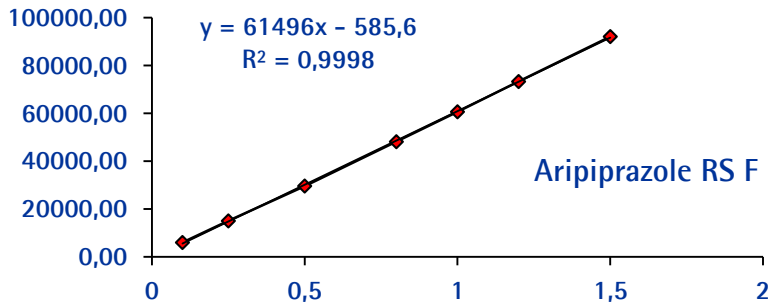
	[Aripiprazole RS F] (ppm)	Area	[Aripiprazole] (ppm)	Area
	0.1	6020	1.0	63167
	0.25	15062	5.0	292127
	0.5	29604	10.0	586674
	0.8	48154	25.0	1487549
	1	60630	50.0	3013178
	1.2	73335	80.0	4878836
	1.5	92098	100.0	6076893
			120.0	7401807
			150.0	9241987
STEYX				35391.1
SLOPE				61651.7
LOD				0.57
LOQ				1.7

Aripiprazole Assay and Related Substances

Validation and Verification Data

3. **Lineratity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting seven (7) concentration levels from 0.1-1.5 ppm of Aripiprazole Related Compound F, and nine (9) concentration levels ranging from 1-150 ppm of Aripiprazole

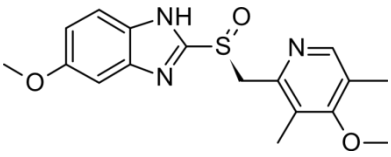




4. **Limit of Quantitation (LOQ) Accuracy**
   Determined by injecting ten (10) standard solutions at LOQ level of Aripiprazole.

	Area Units
1	152629
2	151490
3	152632
4	151973
5	151697
6	151808
7	151794
8	151659
9	152285
10	151524
Mean	151949
STD DEV	424.9
RSD	0.28

Esomeprazole (USP)



Esomeprazole is the S-enantiomer of omeprazole.

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

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

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

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

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

Esomeprazole Magnesium (USP)

Definition:

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

Identification

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

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

Assay: **HPLC**

-Procedure:

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

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

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

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

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

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

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

Esomeprazole Magnesium (USP)

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

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

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

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

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

Content of Magnesium AAS

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

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

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

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

Esomeprazole Magnesium (USP)

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

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

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

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

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

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

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

Esomeprazole Magnesium (USP)

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

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

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

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

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

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

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

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

Esomeprazole Magnesium (USP)

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

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

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

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

Water Determination — Karl Fischer

Method I <921>: 6.0%–8.0%

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

Acceptance criteria: NMT 0.2

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

Esomeprazole Magnesium (USP)

Recommended Merck Millipore products:

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

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

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

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

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

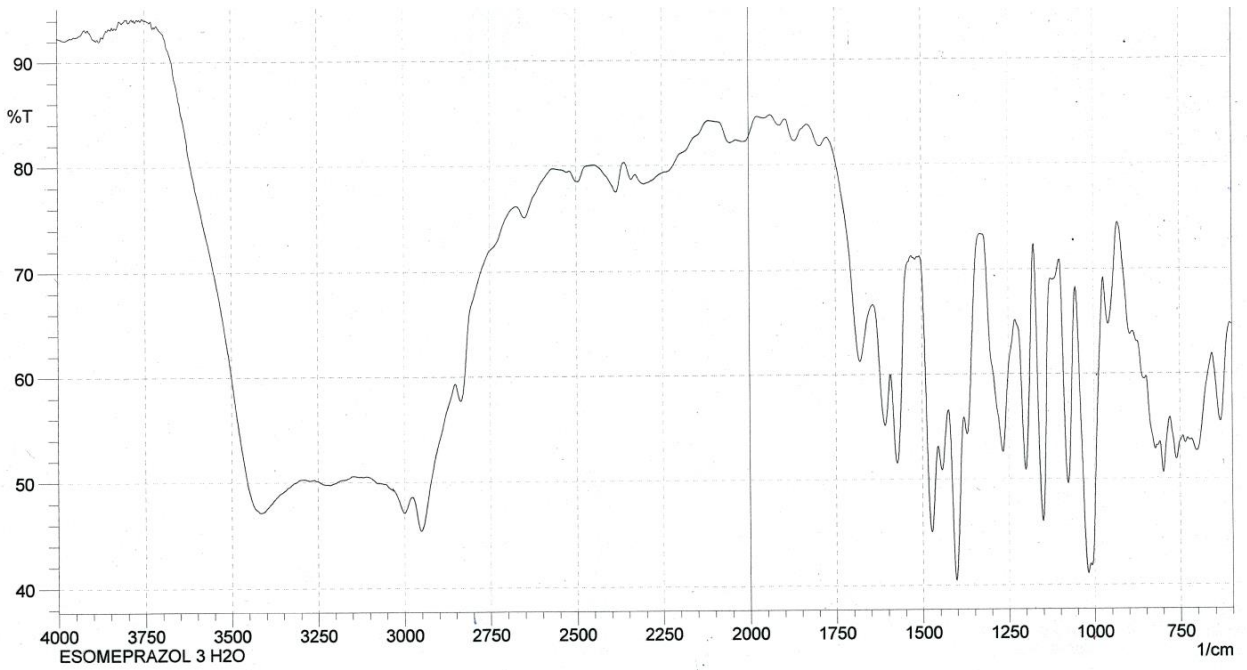
Esomeprazole Magnesium (USP)

Identification (197K)

A. INFRARED ABSORPTION <197K> FTIR

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

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



Esomeprazole Magnesium (USP)

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

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

Absorption at 285.2 → 0.563

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

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

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

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

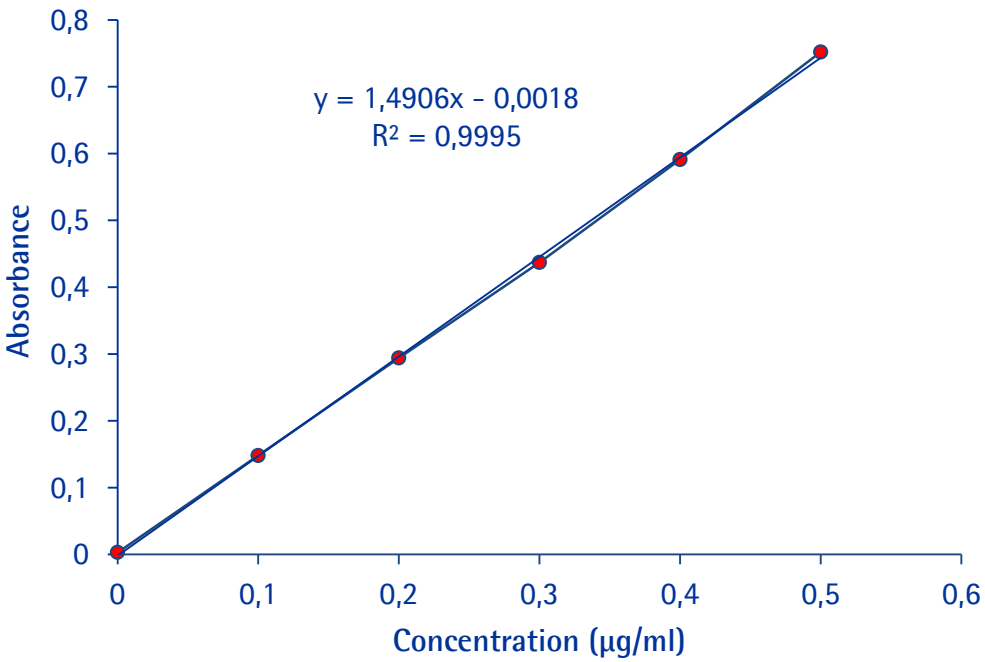
	Solution A	Lanthanum oxide solution	Dilution	Final standard concentration
Standard Solution B	5.0 ml	4.0 ml	100 ml	0.1 µg / ml
Standard Solution C	10.0 ml	4.0 ml	100 ml	0.2 µg / ml
Standard Solution D	15.0 ml	4.0 ml	100 ml	0.3 µg / ml
Standard Solution E	20.0 ml	4.0 ml	100 ml	0.4 µg / ml
Standard Solution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

Esomeprazole Magnesium (USP)

Calibration Curve (AAS):

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

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



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

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

Esomeprazole Magnesium (USP)

Water Determination <USP 921>

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

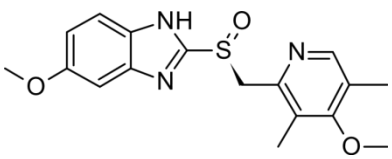
In the case of Esomeprazole the water determination can be carried out without problems according to standard methods.

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

Titration one component system

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

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



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

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

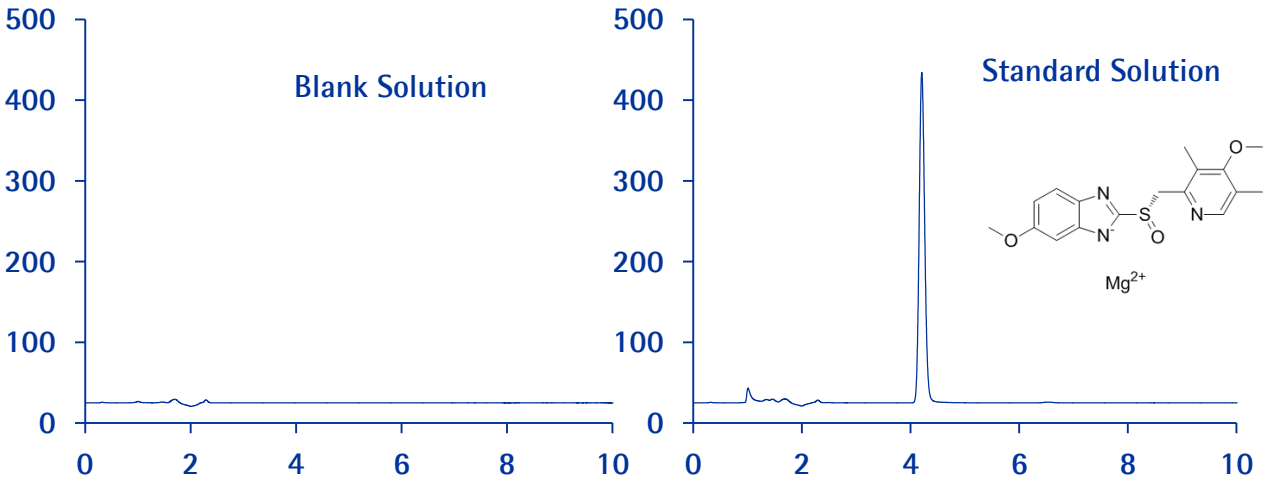
Product	P/N
CombiTitrant 5 one-component reagent for volumetric KF titration 1 ml = ca. 5 mg H2O apura®	1.88005
CombiSolvent methanol-free solvent for volumetric KF titration with one component reagents apura®	1.88008

Esomeprazole Magnesium (USP) – Assay

Purospher STAR® RP-8 endcapped HPLC

Chromatographic Conditions

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



System Suitability requirement: Column efficiency: NLT 2000 theoretical plates

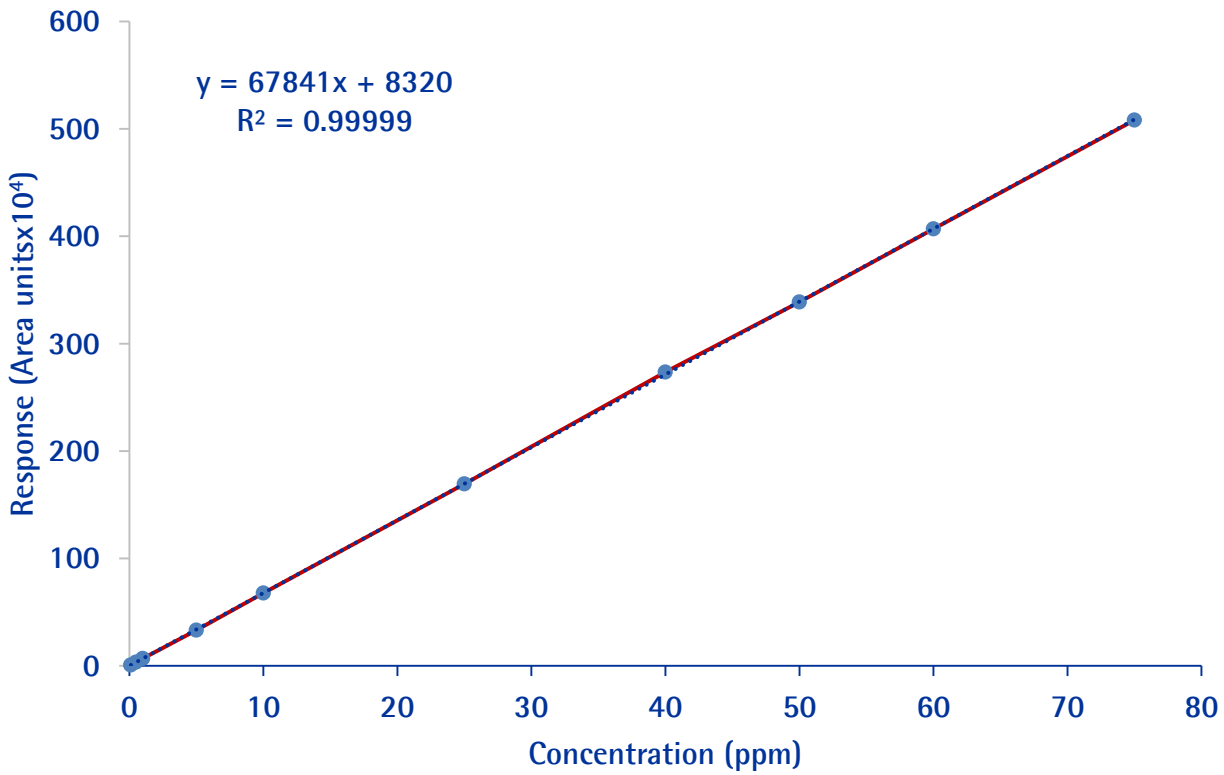
Chromatographic Data:

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

Esomeprazole Magnesium (USP)

Linearity:

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

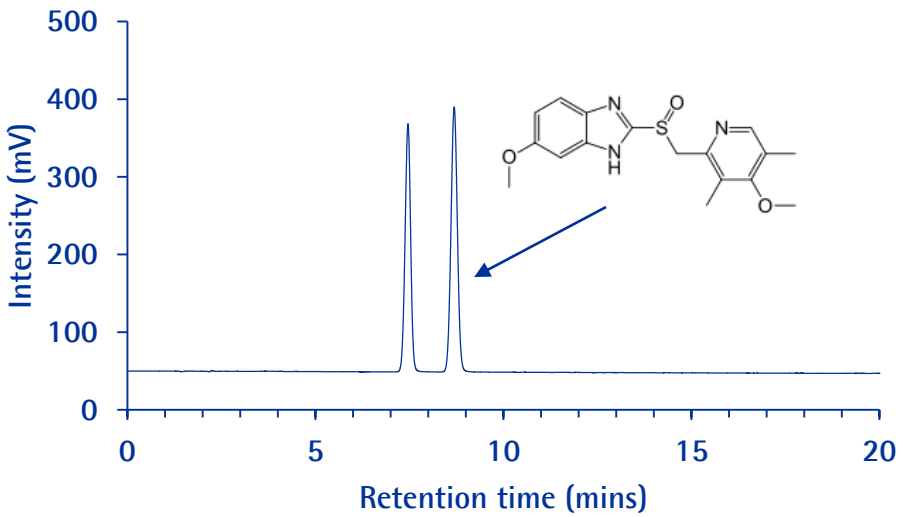


Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped HPLC

Chromatographic Conditions

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



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

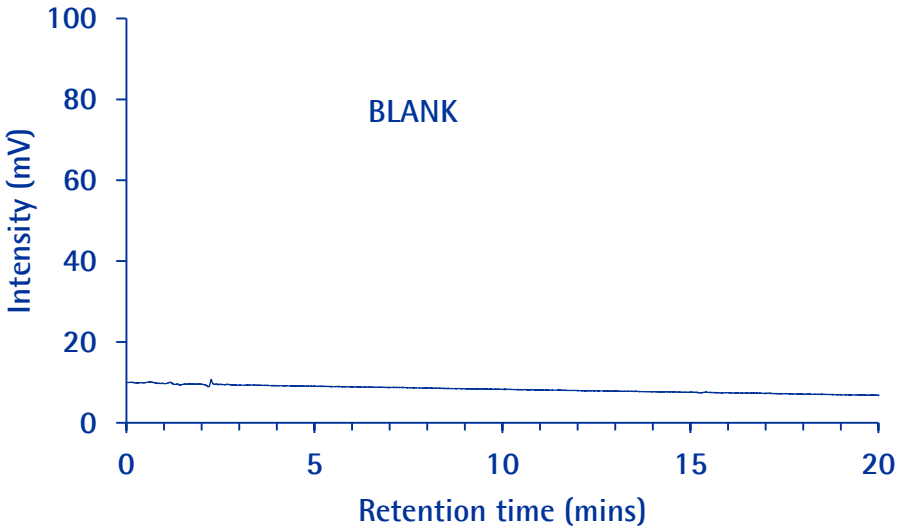
Chromatographic Data : SST solution

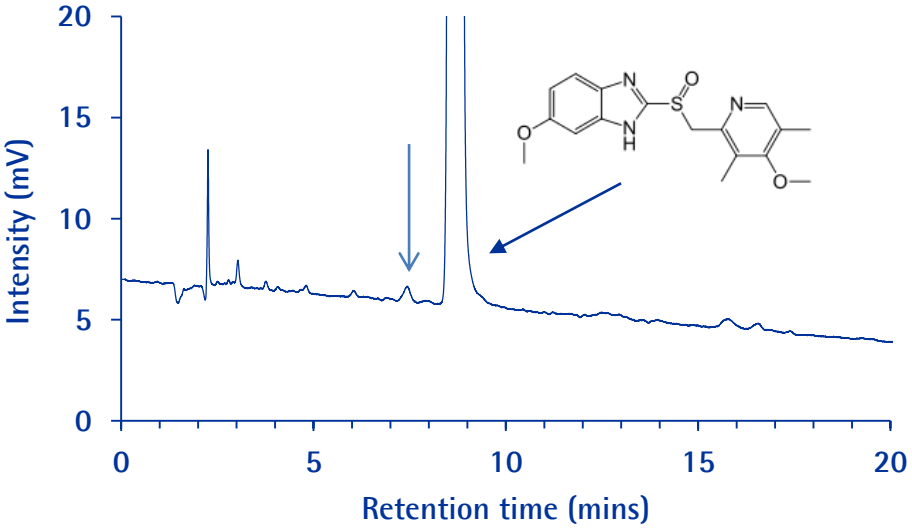
No.	Compound	Retention Time (min)	Resolution	RRT
1	Omeprazole Related compound A	7.46	-	0.85
2	Omeprazole	8.69	4.2	1.00

Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped

HPLC





Chromatographic Data : SST solution

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

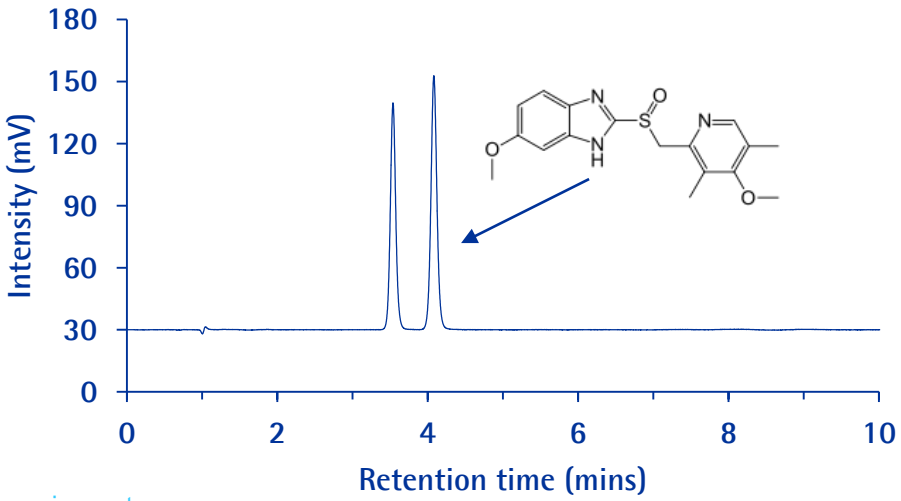
Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped

UHPLC

Chromatographic Conditions

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



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

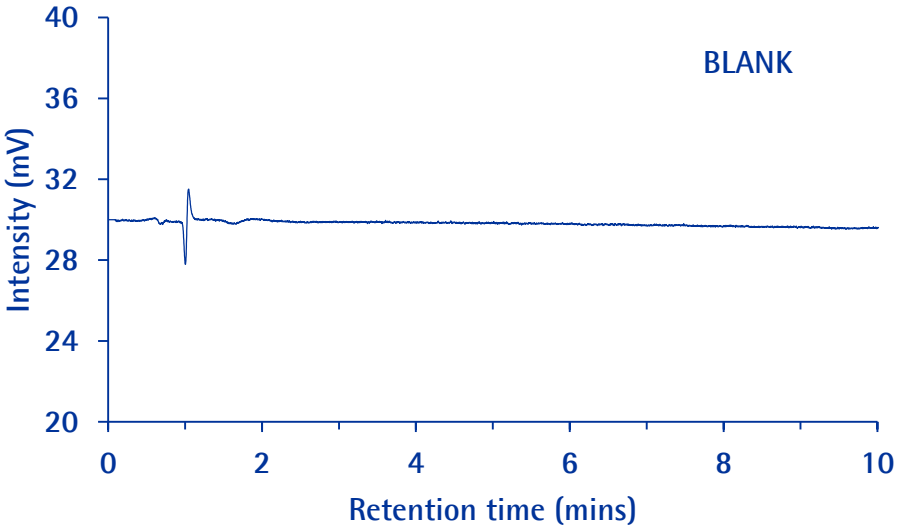
Chromatographic Data : SST solution

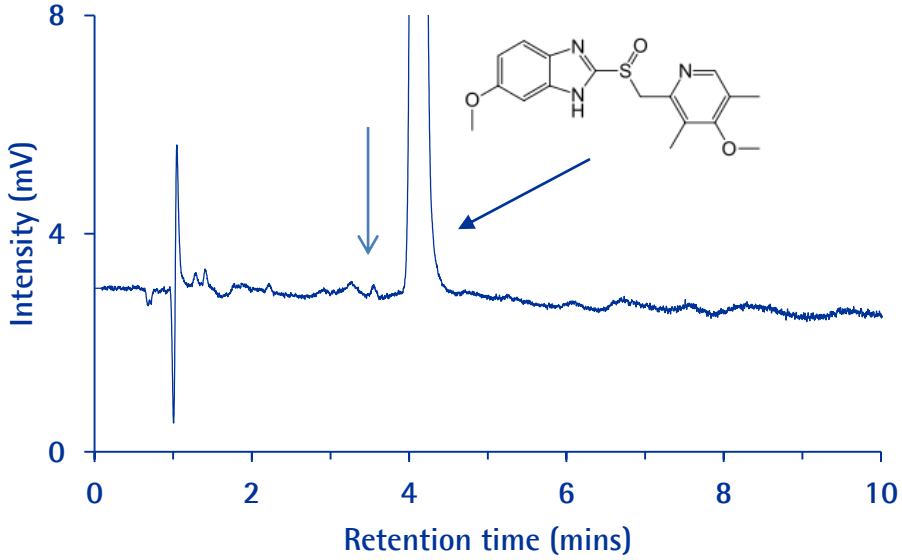
No.	Compound	Retention Time (min)	Resolution	RRT
1	Related compound A	3.5	-	0.85
2	Omeprazole	4.1	4.2	1.00

Esomeprazole Related Substances (USP)

Purospher STAR® RP-8 endcapped

UHPLC





Chromatographic Data : SST solution

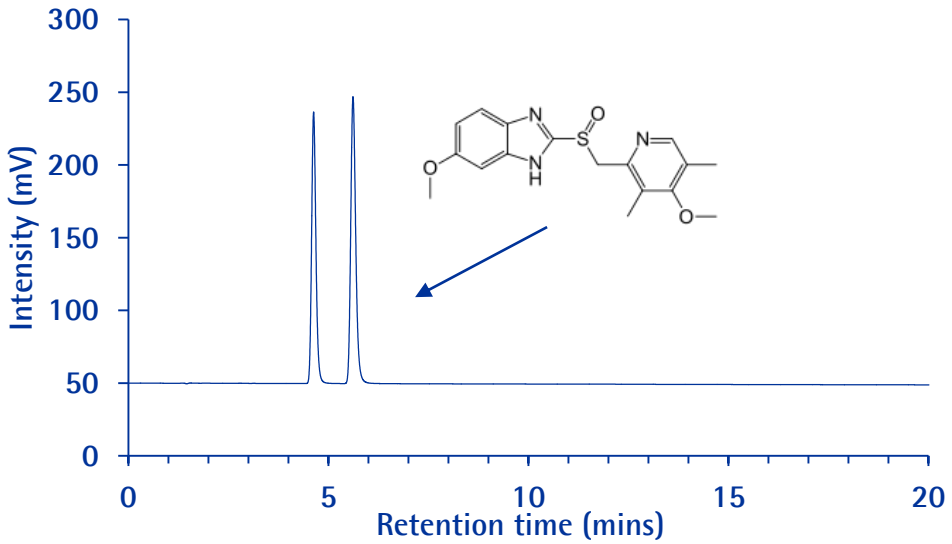
No.	Compound	Retention Time (min)	Resolution	RRT
1	Related compound A	3.5	-	0.85
2	Omeprazole	4.1	4.2	1.00

Esomeprazole Related Substances (USP)

Chromolith HighResolution® RP-18 endcapped HPLC

Chromatographic Conditions

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



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

Chromatographic Data : SST solution

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

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

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

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

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

Diluent: Prepare as directed in Identification test A.

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

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

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

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

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

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

System suitability
Sample: Standard solution

Suitability requirements
Relative standard deviation: NMT 2.0%

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

Result = (rU/rS) × (CS/CU) × 100

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

Acceptance criteria: 90.0%–110.0%

Dissolution <711> HPLC

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

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

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

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

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

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

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

Result = (rU/rS) × (CS/L) ×V× 100

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

IMPURITIES - Organic Impurities **HPLC**

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

Mobile phase: See Table.

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

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

Esomeprazole Magnesium (USP)
– Delayed Release Capsules

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

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

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

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

Result = (rU/rT) × 100

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

Acceptance criteria: See Table.

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

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

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

Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities

Chromatographic Conditions

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

Injection: 20 µL

Detection: UV 302 nm

Cell: 10 µL

Flow Rate: 1.0 mL/min

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

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

Gradient: See table

Temperature: 25°C

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

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

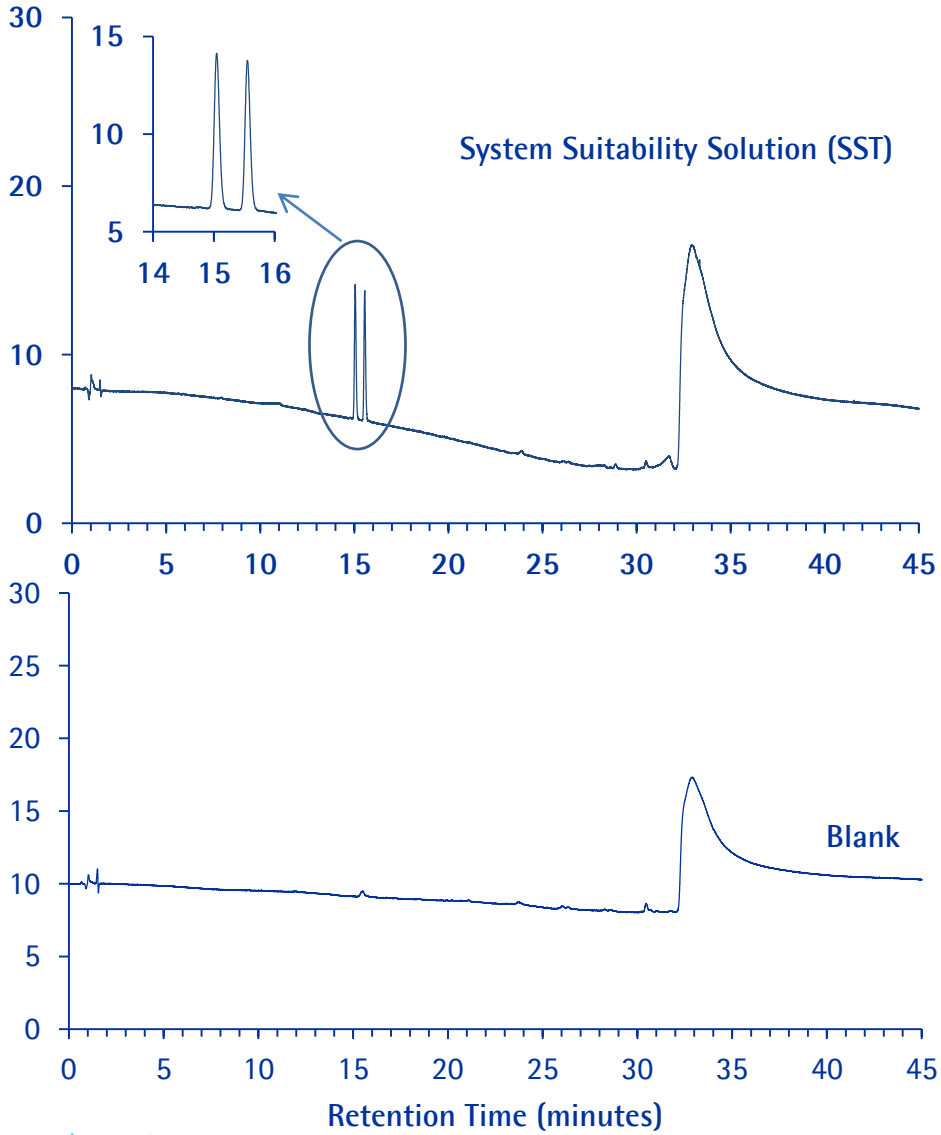
Standard Solution: 1 µg/mL each of USP Omeprazole and USP Omeprazole Related Compound A in methanol

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

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

Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities



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

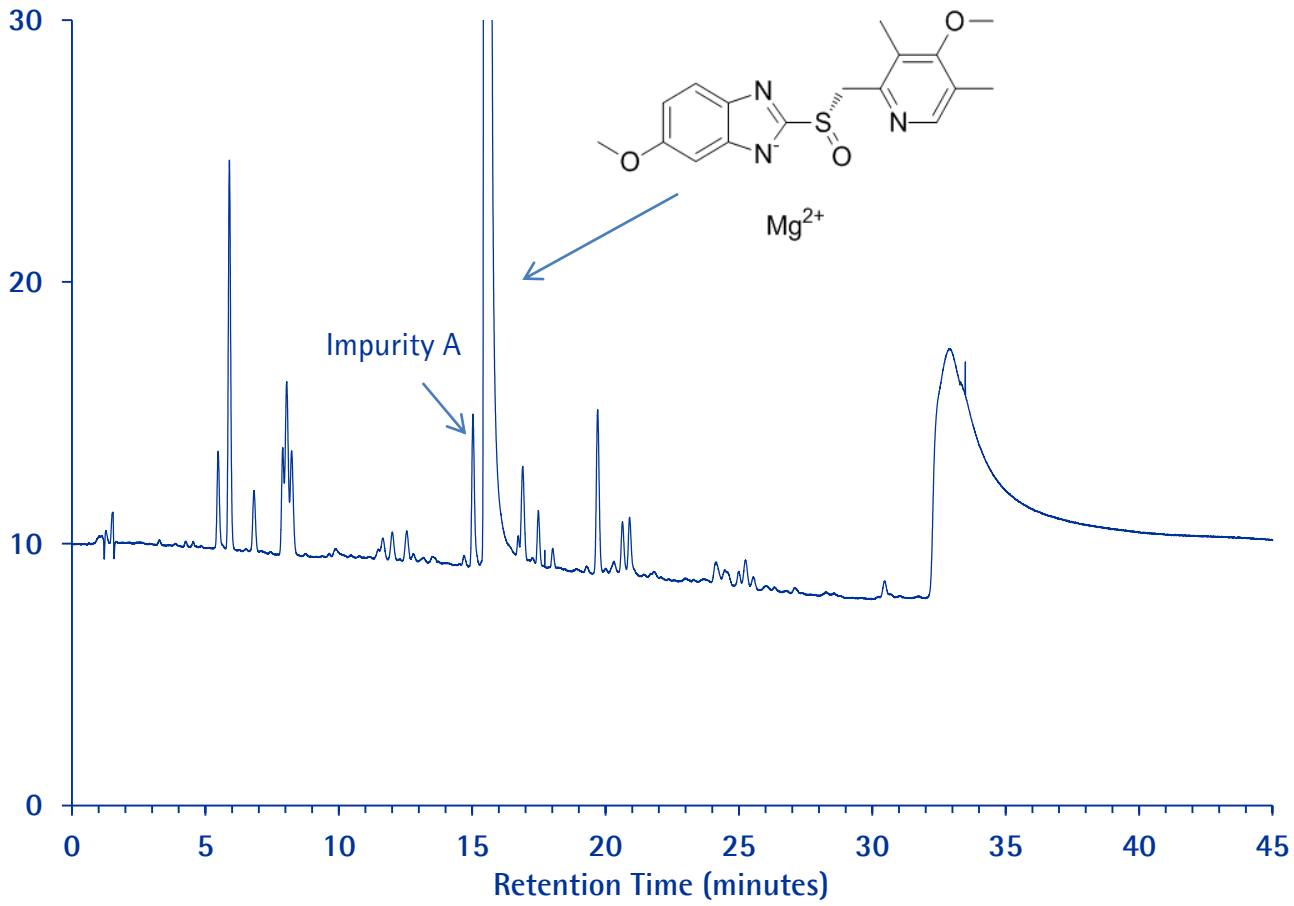
Chromatographic Data:

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

Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities

Sample Analysis (delayed release capsules)



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

Chromatographic Data:

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

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

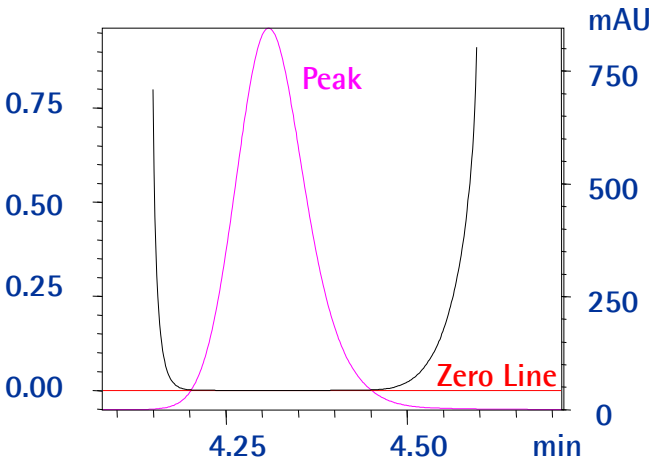
Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Dissolution

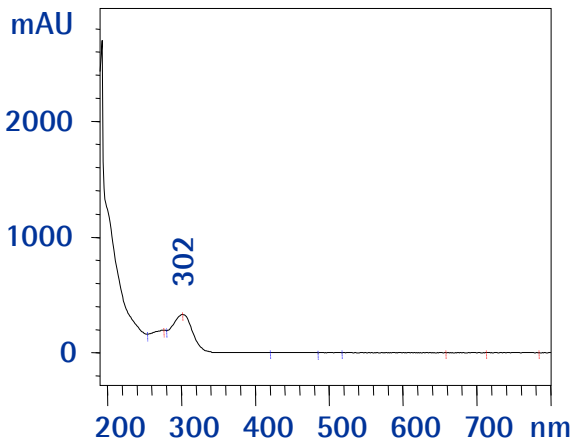
Chromatographic Conditions

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

Peak Purity

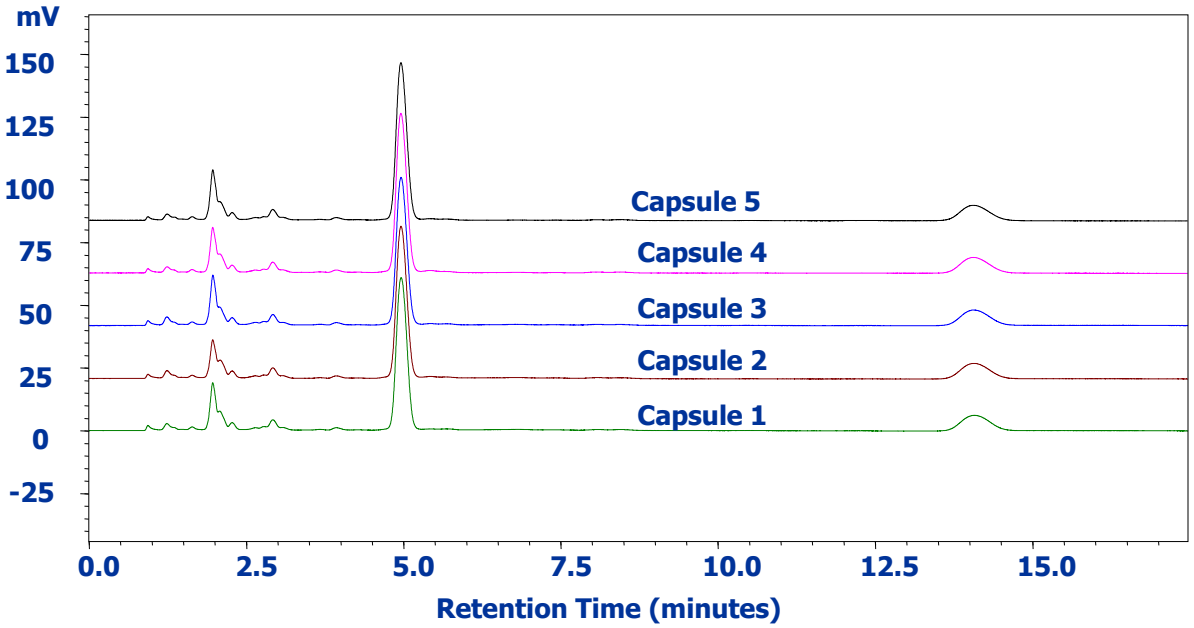


UV Analysis



Esomeprazole Magnesium Capsules (USP)

Purospher STAR® RP-18 endcapped – Related Impurities



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

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

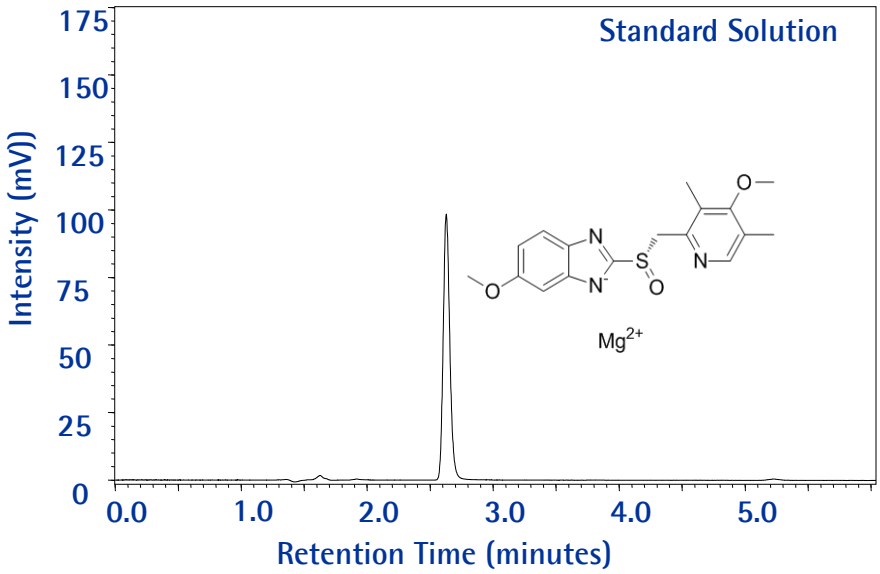
rU = peak response from the Sample solution
rS = peak response from the Standard solution
CS = concentration of the Standard solution (mg/mL)
L = label claim (mg/Capsule)
V = volume of Medium, 1000 mL
Acceptance criteria: NLT 75% of the claimed esomeprazole ($C_{17}H_{19}N_3O_3S$) is dissolved.

Esomeprazole Magnesium Capsules (USP)

Chromolith® RP-18 endcapped – Related Impurities

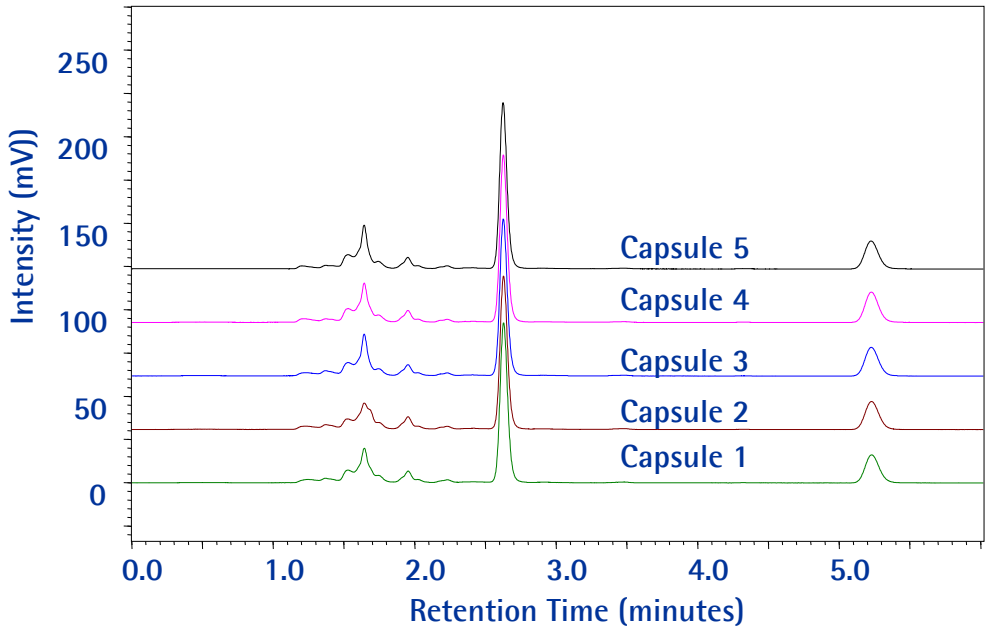
Chromatographic Conditions

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



Esomeprazole Magnesium Capsules (USP)

Chromolith® RP-18 endcapped – Related Impurities



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

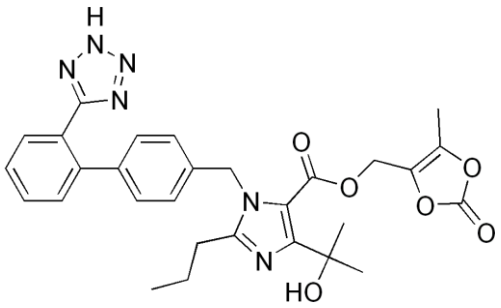
Calculate the percentage of esomeprazole dissolved: Result = (rU/rS) × (CS/L) ×V× 100 = 90.2%

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

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

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

Olmesartan Medoxomil (USP)



Olmesartan medoxomil is an angiotensin II receptor antagonist.
It is an ester prodrug that is completely and rapidly hydrolyzed to the active acid form, olmesartan. It is used to treat high blood pressure.

Olmesartan medoxomil was developed by Daichii Sankyo in 1995.

Common commercial brand name: Benicar (US), Olmetec (EU, Canada and Japan), WinBP, Olsar, Golme (India) etc. Sales in 2010 were $2.5 billion globally.
Patent expiry in 2016

In this application compilation, we have followed the experimental conditions in USP37-NF32 for Olmesartan medoxomil.

Identification – FTIR (197K)
Assay – HPLC and UHPLC (isocratic methods)
Related Substances (RS) – HPLC (gradient method)
Karl Fischer – water content

The assay and RS methods have been carried out with HPLC using RP-8 and RP-18 endcapped columns. The assay method was, in addition, scaled to two shorter column dimensions with different particle sizes (3 and 2 µm particles).

Finally we have also included a new proposal for UHPLC analysis of olmesartan medoxomil related substances using LC-MS conditions (in-house method), and a proposal for heavy metal analysis per suggestions in the new general chapters USP 232/233 that will come active in 2018 using ICP-OES or ICP-MS analysis.

Olmesartan medoxomil (USP)

Definition:

Olmesartan Medoxomil contains NLT 98.5% and NMT 101.5% of $C_{29}H_{30}N_6O_6$, calculated on the anhydrous and solvent-free basis.

Identification: FTIR

A. Infrared Absorption 197K
B. The ratio of the retention time of the major peak to that of the internal standard of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

Assay: HPLC

Procedure
[Note—The Standard solution and Sample solution are stable for 24 h at 5.]

Diluted phosphoric acid: 0.2% phosphoric acid
Buffer: 0.015 M monobasic potassium phosphate. Adjust the solution with Diluted phosphoric acid (w/v) to a pH of 3.4.
Mobile phase: Acetonitrile and Buffer (17:33)
Diluent 1: Acetonitrile and water (4:1)
Diluent 2: Acetonitrile and water (2:3)
Internal standard solution: 0.5 mg/mL of 4-hydroxybenzoic acid isobutyl ester in Diluent 2.
[Note—This solution is stable for 1 month at room temperature.]
Standard stock solution: 1 mg/mL of USP Olmesartan Medoxomil RS in Diluent 1
Standard solution: 0.05 mg/mL of USP Olmesartan Medoxomil RS from the Standard stock solution and 0.025 mg/mL of p-hydroxybenzoic acid isobutyl ester from the Internal standard solution in Diluent 2
Sample stock solution: 1 mg/mL of Olmesartan Medoxomil in Diluent 1
Sample solution: 0.05 mg/mL of Olmesartan Medoxomil from the Sample stock solution and 0.025 mg/mL of p-hydroxybenzoic acid isobutyl ester from the Internal standard solution in Diluent 2

Chromatographic system (See Chromatography 621, System Suitability.)

Detector: UV 250 nm
Column: 4.6-mm × 15-cm; 5 µm packing L1
Column temperature: 40
Flow rate: 1 mL/min
Injection size: 10 µL

We have used: Purospher® STAR 5µm RP-18 endcapped 150x4.6 mm (1.51455)

Olmesartan medoxomil (USP)

System suitability
Sample: Standard solution

Suitability requirements
Resolution: NLT 4 between olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester
Relative standard deviation: NMT 0.5% for the peak ratio of olmesartan medoxomil and the internal standard

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of olmesartan medoxomil in the portion taken:

Result = (RU/RS) × (CS/CU) × 100

RU = ratio of the peak areas of olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester from the Sample solution
RS = ratio of the peak areas of olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester from the Standard solution
CS = concentration of USP Olmesartan Medoxomil RS in the Standard solution (mg/mL)
CU = concentration of Olmesartan Medoxomil in the Sample solution (mg/mL)

Acceptance criteria: 98.5%–101.5% on the anhydrous and solvent-free basis

IMPURITIES:

Inorganic Impurities
- Residue on Ignition 281: NMT 0.1%. [Note—The ignition temperature range is 450 to 550.]
- Heavy Metals, Method II231: NMT 10 ppm

Organic Impurities
Procedure
Buffer: Prepare as directed in the Assay.
Solution A: Acetonitrile and Buffer (1:4)
Solution B: Acetonitrile and Buffer (4:1)
Mobile phase: See the gradient table.

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	75	25
35	0	100
45	0	100

System suitability solution: 0.01 mg/mL each of USP Olmesartan Medoxomil RS and USP Olmesartan Medoxomil Related Compound A RS in acetonitrile
Standard solution: 0.01 mg/mL of USP Olmesartan Medoxomil RS in acetonitrile
Sample solution: 1 mg/mL of Olmesartan Medoxomil in acetonitrile

Olmesartan medoxomil (USP)

Chromatographic system (See Chromatography 621, System Suitability.)
[Note—A guard column of 4.6-mm × 5-cm of packing L7 may be used.]

Detector: UV 250 nm
Column: 4.6-mm × 10-cm; 3.5 µm packing L7
Column temperature: 40
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Suitability requirements
Sample: System suitability solution

Resolution: NLT 5 between olmesartan medoxomil and olmesartan medoxomil related compound A
Relative standard deviation: NMT 2.0% for the olmesartan medoxomil peak

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of each impurity in the portion of Olmesartan Medoxomil taken:

Result = (rU/rS) × (CS/CU) × (1/F) × 100

rU = peak response of each impurity from the Sample solution
rS = peak response of olmesartan medoxomil from the Standard solution
CS = concentration of USP Olmesartan Medoxomil RS in the Standard solution (mg/mL)
CU = concentration of Olmesartan Medoxomil in the Sample solution (mg/mL)
F = relative response factor (see the Impurity Table)

Acceptance criteria
Individual impurities: See the Impurity Table on the next page.
Total impurities: NMT 1.3%. *[Note—Disregard any peak below 0.05%.]*

Olmesartan medoxomil (USP)

Name	RRT	RRF	Acceptance Criteria, NMT (%)
Olmesartan[a]	0.2	1.0	0.5
Olmesartan medoxomil related compound A[b]	0.7	1.6	0.1
Olmesartan medoxomil	1.0	1.0	-
Olefinic impurity[c]	1.6	1.0	0.6
N-alkyl impurity[d]	3.4	0.7	0.1
Any other individual unidentified impurity	-	1.0	0.1

a 1-{[2¢-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4-(2-hydroxypropan-2-yl)-2-propyl-1H-imidazole-5-carboxylic acid.
b 1-{[2¢-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1H-furo[3,4-d]imidazol-6(4H)-one.
c (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 1-((2¢-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl)-4-(prop-1-en-2-yl)-2-propyl-1H- imidazole-5-carboxylate.
d (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-((2¢-(2-trityl-1H-tetrazol-5-yl)biphenyl-4-yl)methyl)-1H-imidazole-5-carboxylate

SPECIFIC TESTS

Limit of Acetone (if present) – Not conducted because we only performed analysis of USP reference standards

Water Determination Method 921-Ic: NMT 0.5%

ADDITIONAL REQUIREMENTS

Packaging and Storage: Preserve in well-closed containers, protect from moisture, and store below 25.

USP Reference Standards
USP Olmesartan Medoxomil RS
USP Olmesartan Medoxomil Related Compound A RS
1-{[2¢-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1H-furo[3,4-d]imidazol-6(4H)-one.

Olmesartan medoxomil (USP)

Recommended Merck Millipore products:

FTIR – Identification (197K)
Potassium bromide for IR spectroscopy Uvasol® (1.04907)
KF – Water Determination (921 –Ia)
CombiCoulomat fritless Karl Fischer reagent for coulometric water determination for cells with and without diaphragm apura® 1.88002
CombiCoulomat fritless - Coulometric KF reagent for cells with or without diaphragm; 100 mL (1.09257)

HPLC Assay and Related Substances
Purospher® STAR RP-18 endcapped (5 µm) 150x4.6 mm (1.51455) for assay scaled to
Purospher® STAR RP-18 endcapped (3 µm) 100x2.1mm (1.50653) for assay
Purospher® STAR RP-18 endcapped (2 µm) 50x2.1mm (1.50651) for assay
Purospher ® STAR RP-8 endcapped (3 µm) 100x4.6 mm for RS analysis (1.50013.7220) for RS analysis
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur 106342
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur 100573
Acetonitrile (isocratic grade for liquid chromatography LiChrosolv® 1.14291)
Acetonitrile (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur 1.00030
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)

LC-MS - Related Substances (proposal method)
Purospher® STAR RP-18 endcapped (3 µm) 100x2.1mm (1.50653)
Acetonitrile hypergrade for LC-MS LiChrosolv® 100029
Formic acid 98-100% for analysis EMSURE® ACS,Reag. Ph Eur 100264
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)

ICP Analysis
Nitric acid 65% Suprapur® (1.00441)
Hydrochloric acid 30% Suprapur® (1.00318)
Hydrogen peroxide 30% Suprapur® (1.07298)

Elements: As, Cd, Cu, Hg, Mo, Ni, Pb, V
ICP Multi-element standard USP-I according to USP <232> oral dose. Certipur® (5.05101)
ICP Multi-element standard USP-II according to USP <232> parenteral dose. Certipur® (5.05102)

Elements: Ir, Os, Pd, Pt, Rh, Ru
ICP Multi-element standard USP-III according to USP <232> oral dose 100 mg/l. Certipur® (5.05103)
ICP Multi-element standard USP-IV according to USP <232> parenteral dose 10 mg/.Certipur® (5.05104)

Olmesartan medoxomil (USP)

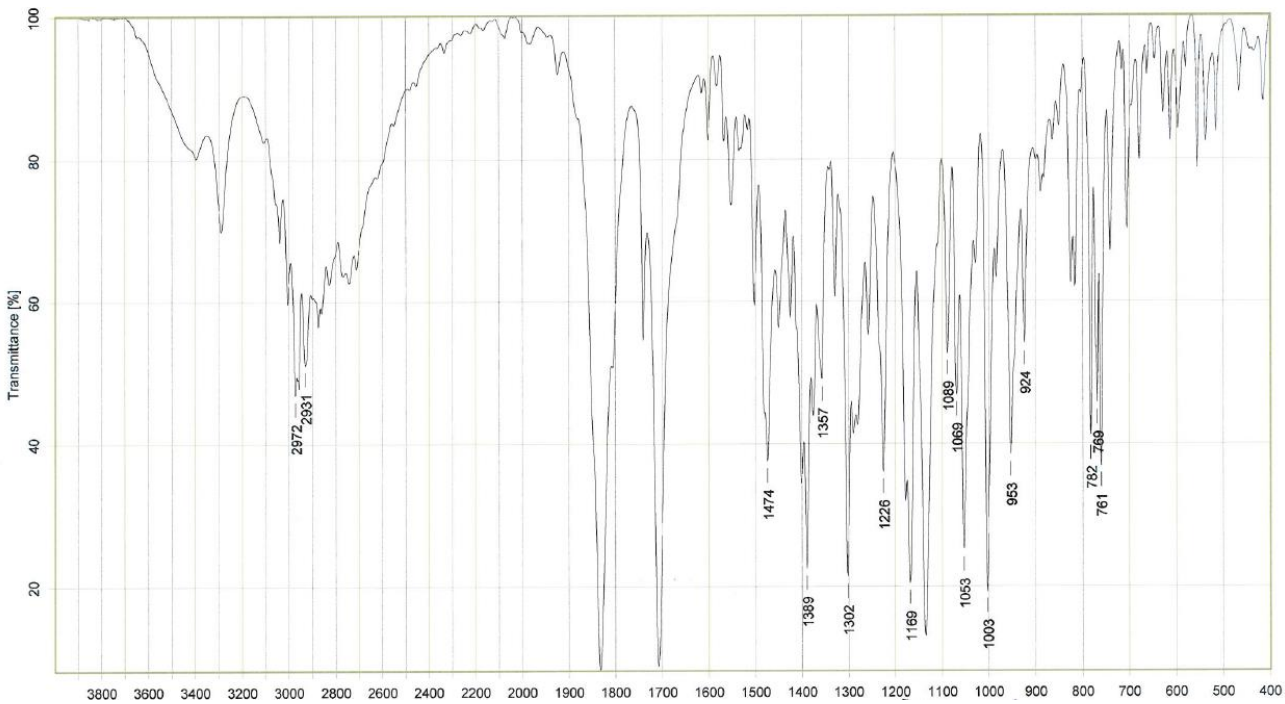
Identification

A. INFRARED ABSORPTION <197K> FTIR

The reference 197K in a monograph signifies that the substance under examination is mixed intimately with potassium bromide.
We recommend Potassium bromide for IR spectroscopy Uvasol® (1.04907) to be used.

B. HPLC (Assay)

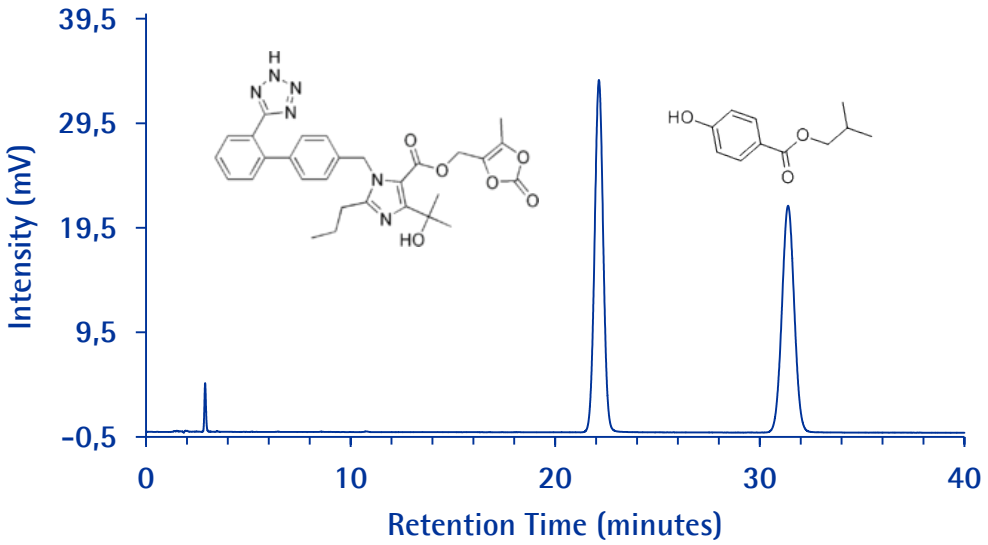
The ratio of the retention time of the major peak to that of the internal standard of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.



Olmesartan medoxomil (USP)

Purospher® STAR RP-18 endcapped HPLC - Assay

Column:	Purospher ® STAR RP-18 endcapped (5µm) 150x4.6 mm	1.51455.0001
Injection:	10 µL	
Detection:	UV, 250nm	
Cell:	11 µL	
Flow Rate:	1mL/min	
Solution A:	15mM monobasic potassium phosphate pH=3.4	
Solution B:	Acetonitrile (Gradient Grade 1.00030)	
Mobile Phase:	Buffer and acetonitrile 33:17 (v/v)	
Temperature:	40°C	
Diluent 1	Acetonitrile:Water 4:1	
Diluent 2	Acetonitrile:Water 2:3	
Standard solution:	0.05mg/mL of Olmesartan medoxomil RS of the Standard Stock solution and 0.025mg/mL of p-Hydroxybenzoic acid isobutyl ester from the internal Standard solution in Diluent 2	
Standard Stock soln.	1mg/mL of Olmesartan medoxomil RS in Diluent 1	
Internal Standard	0.5mg/mL of p-Hydroxybenzoic acid isobutyl ester in Diluent 2	
Pressure Drop:	63 Bar (907 psi)	



System Suitability criteria:
Resolution: NLT 4 between Olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester

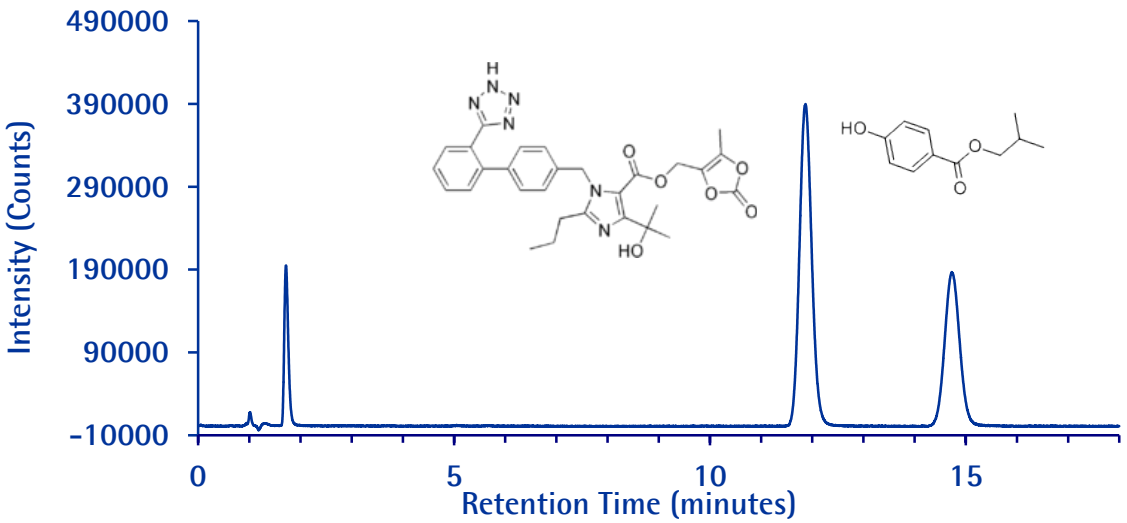
Chromatographic Data: (Standard solution)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	2.9			
Olmesartan RS	22.1		13101	1.02
p-HBA i-But ester	31.3	9.8	12822	1.01

Olmesartan medoxomil (USP)

Purospher® STAR RP-18 endcapped HPLC - Assay

Column:	Purospher® STAR RP-18 endcapped (3 µm) 100x2.1mm	1.50653.0001
Injection:	2.1 µL	
Detection:	UV; 250nm	
Cell:	11 µL	
Flow Rate:	1mL/min	
Solution A:	15mM monobasic potassium phosphate pH=3.4	
Solution B:	Acetonitrile (Gradient Grade 1.00030)	
Mobile Phase:	Buffer and Acetonitrile 33:17 (v/v)	
Temperature:	40°C	
Diluent 1	Acetonitrile:Water 4:1	
Diluent 2	Acetonitrile:Water 2:3	
Standard solution:	0.05mg/mL of Olmesartan medoxomil RS of the Standard Stock solution and 0.025mg/mL of p-Hydroxybenzoic acid isobutyl ester from the internal Standard solution in Diluent 2	
Standard Stock soln:	1mg/mL of Olmesartan medoxomil RS in Diluent 1	
Internal Standard	0.5mg/mL of p-Hydroxybenzoic acid isobutyl ester in Diluent 2	
Pressure Drop:	76 Bar (1102 psi)	



System Suitability criteria:
Resolution: NLT 4 between Olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester

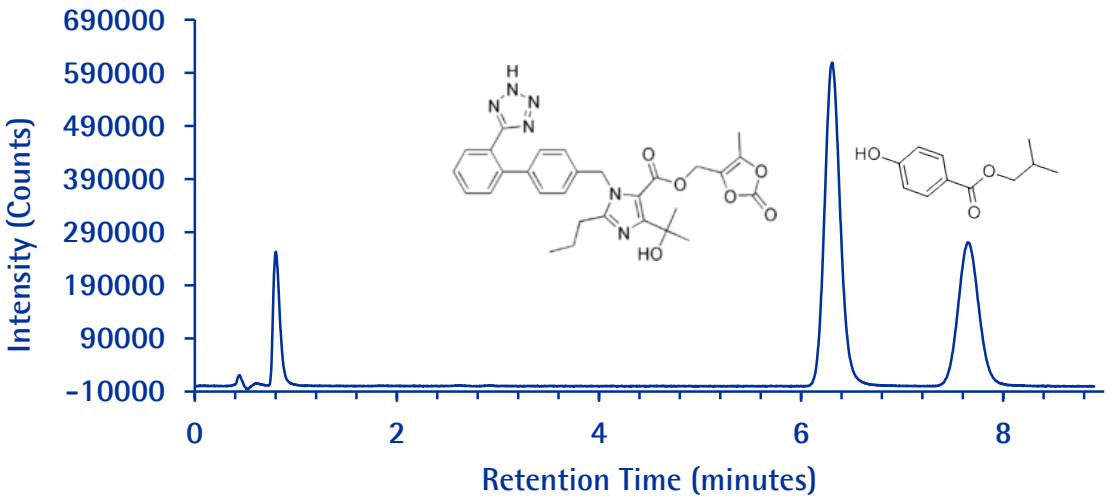
Chromatographic Data: (Standard solution)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	1.3			
Olmesartan RS	11.9		11270	1.10
p-HBA i-But ester	14.7	5.7	11345	1.08

Olmesartan medoxomil (USP)

Purospher® STAR RP-18 endcapped UHPLC - Assay

Column:	Purospher® STAR RP-18 endcapped (2 µm) 50x2.1mm	1.50651.0001
Injection:	2.1 µL	
Detection:	UV; 250nm	
Cell:	1.4 µL	
Flow Rate:	0.21mL/min	
Solution A:	15mM monobasic potassium phosphate pH=3.4	
Solution B:	Acetonitrile (Gradient Grade 1.00030)	
Mobile Phase:	Buffer and Acetonitrile 33:17 (v/v)	
Temperature:	40°C	
Diluent 1	Acetonitrile:Water 4:1	
Diluent 2	Acetonitrile:Water 2:3	
Standard solution:	0.05mg/mL of Olmesartan medoxomil RS of the Standard Stock solution and 0.025mg/mL of p-Hydroxybenzoic acid isobutyl ester from the internal Standard solution in Diluent 2	
Standard Stock soln:	1mg/mL of Olmesartan medoxomil RS in Diluent 1	
Internal Standard	0.5mg/mL of p-Hydroxybenzoic acid isobutyl ester in Diluent 2	
Pressure Drop:	48 Bar (696 psi)	



System Suitability criteria:
Resolution: NLT 4 between Olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester

Chromatographic Data: (Standard solution)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	0.7			
Olmesartan RS	6.4		7962	1.13
p-HBA i-But ester	7.8	4.0	6527	1.09

Olmesartan medoxomil (USP)

Validation and Verification HPLC – Assay

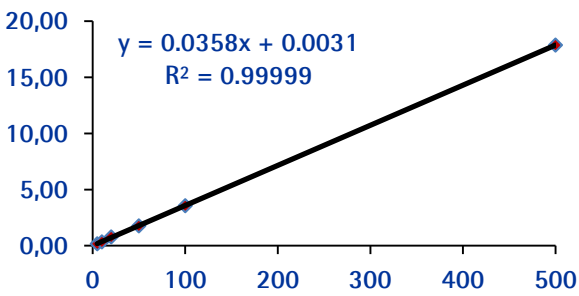
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Olmesartan medoxomil RS A and Olmesartan medoxomil RS using a Purospher® STAR RP-18 endcapped (5µm) 150x4.6 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Olmesartan medoxomil	22.1	-	1.0	-
p-HBA i-Butyl ester	31.4	0.70	1.0	9.8

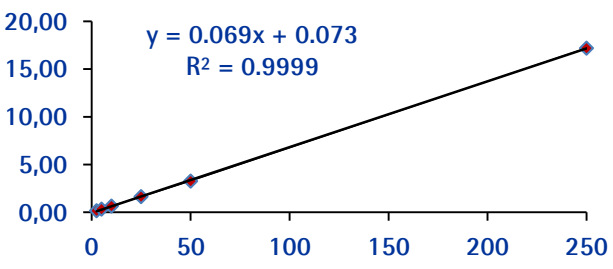
2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 5-500 ppm of Olmesartan medoxomil RS, and six (6) concentration levels ranging from 2.5-250 ppm of p-Hydroxybenzoic acid isobutyl ester

	[Olmesartan medoxomil] (ppm)	Area (mAU*min)	[p-HBA i-Butyl ester] (ppm)	Area (mAU*min)
	5	0.17	2.5	0.15
	10	0.35	5.0	0.33
	20	0.79	10	0.65
	50	1.77	25	1.63
	100	3.56	50	3.26
	500	17.88	250	17.20
STEYX		0.0031		0.0734
SLOPE		0.0358		0.0690
LOD		3.5		3.5
LOQ		10.7		10.7

Olmesartan medoxomil



p-Hydroxybenzoic acid isobutyl ester

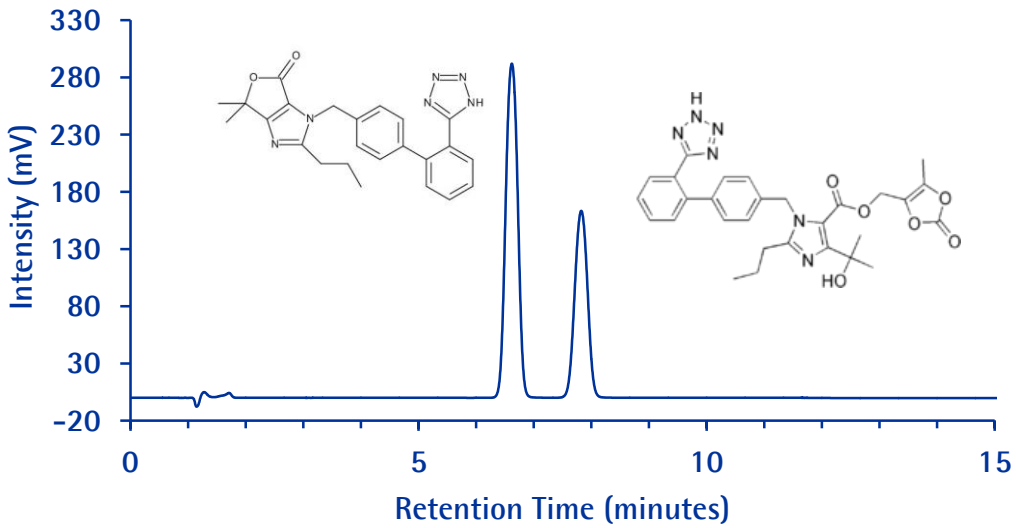


Olmesartan medoxomil (USP)

Purospher® STAR RP-18 endcapped HPLC – RS

Column:	Purospher® STAR RP-8 endcapped (3 µm) 100x4.6 mm	1.50013.7220
Injection:	10 µL	
Detection:	UV; 250nm	
Cell:	11 µL	
Flow Rate:	1mL/min	
Solution A:	15mM monobasic potassium phosphate pH=3.4	
Solution B:	Acetonitrile (Gradient Grade 1.00030)	
Mobile Phase:	A : Solution A : Solution B 4:1 (v:v) B : Solution A : Solution B 1:4 (v:v)	
Gradient:	See table	
Temperature:	40°C	
Diluent	Acetonitrile	
Standard for Impurity	0.01mg/mL of Olmesartan medoxomil RS in Acetonitrile	
Sample for Impurity	1mg/mL of Olmesartan medoxomil RS in Acetonitrile	
SST for Impurity	0.01mg/mL each of Olmesartan medoxomil RS and related compound A in Acetonitrile	
Pressure Drop:	48-108 Bar (696-1566psi)	

Time (min)	A (%)	B (%)
0	75	25
10	75	25
35	0	100
45	0	100



System Suitability criteria:
Resolution: NLT 5 between Olmesartan medoxomil and Olmesartan medoxomil RS A

Chromatographic Data: (SST solution)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	1.3			
Olmesartan RS A	6.6		5004	1.00
Olmesartan RS	7.8	5.7	5926	1.00

Olmesartan medoxomil (USP)

Validation and Verification HPLC – RS

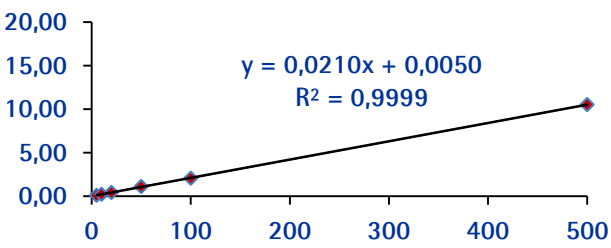
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Olmesartan medoxomil RS A and Olmesartan medoxomil RS using a Purospher® STAR RP-8 endcapped (3 µm) 100x4.6 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Olmesartan medoxomil RS A	6.6	-	1.0	-
Olmesartan medoxomil	7.8	0.85	1.0	5.7

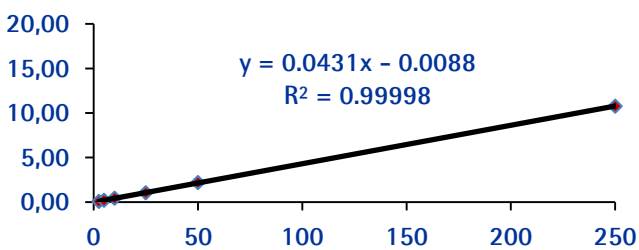
2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 5-500 ppm of Olmesartan medoxomil RS, and six (6) concentration levels ranging from 2.5-250 ppm of Olmesartan medoxomil RS A

	[Olmesartan medoxomil] (ppm)	Area (mAU*min)	[Olmesartan medoxomil RS A] (ppm)	Area (mAU*min)
	5	0.10	2.5	0.08
	10	0.21	5.0	0.21
	20	0.43	10	0.43
	50	1.10	25	1.05
	100	2.07	50	2.19
	500	10.51	250	10.77
STEYX		0.0050		-0.0088
SLOPE		0.0210		0.0431
LOD		5.1		2.0
LOQ		15.3		6.2

Olmesartan medoxomil



Olmesartan medoxomil RS A



Olmesartan medoxomil (USP)

New LC-MS Compatible Related Substances Method

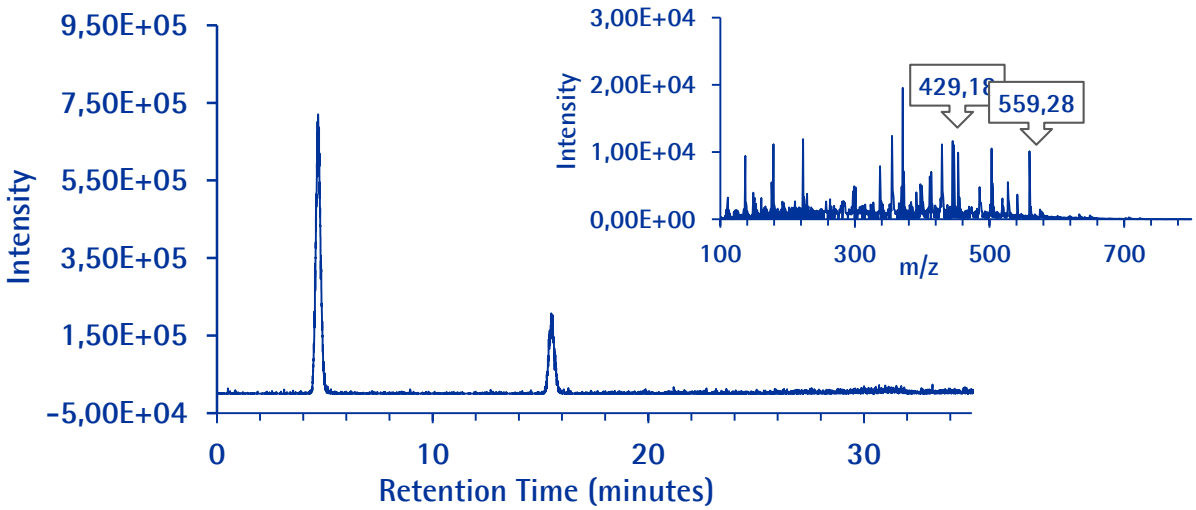
On the following pages, you will find presented a new alternative approach for the analysis of Olmesartan medoxomil and its related substance RS A (*1-{[2¢-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1H-furo[3,4-d]imidazol-6(4H)-one)* using LC-MS. The new procedure is both MS and UV compatible.

Column: Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm 1.50653.0001
Injection: 2 µL
Detection: ESI-(+)-MS (m/z 100-800) Nebu.405 psi, Dry Gas 12L/min, Dry Temp.365°C, Scan mode - normal
Flow Rate: 210 µL/min

Mobile Phase:
Solution A: A : 90% Water + 10% Acetonitrile + 0,1% formic acid
Solution B: B: 10% Water + 90% Acetonitrile + 0,1% formic acid
Gradient: See table
Temperature: 40°C

Time (min)	A (%)	B (%)
0	75	25
10	75	25
35	0	100
45	0	100

Diluent Acetonitrile
SST for Impurity 0.01mg/mL each of Olmesartan medoxomil RS and related compound A in Acetonitrile
Pressure Drop: 51-102 Bar (734-1469 psi)



Chromatographic Data: (SST solution)

Compound	Retention Time (min)	Molecular Weight	m/z
Olmesartan RC A	4.8	428.5	429.2
Olmesartan RS	15.3	558.5	559.4

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

Olmesartan medoxomil (USP)

New LC-MS Compatible Related Substances Method

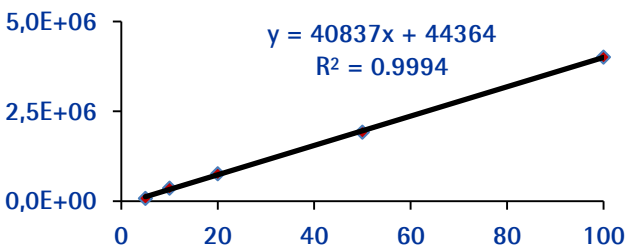
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Olmesartan medoxomil RS A and Olmesartan medoxomil RS using a Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm column.

Compound	Retention Time (min)	Tailing factor	Resolution
Olmesartan medoxomil RS A	4.8	1.1	-
Olmesartan medoxomil	15.3	1.1	>>5

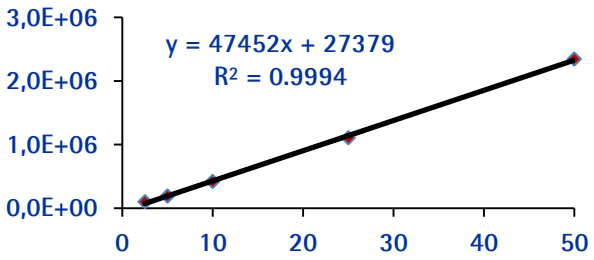
2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 5-500 ppm of Olmesartan medoxomil RS, and six (6) concentration levels ranging from 2.5-250 ppm of Olmesartan medoxomil RS A

	[Olmesartan medoxomil] (ppm)	Counts	[Olmesartan medoxomil RS A] (ppm)	counts
	5	75999	2.5	101188
	10	362596	5.0	191738
	20	764244	10	420902
	50	1920491	25	1108372
	100	4009862	50	2345859
STEYX		44364		27379
SLOPE		40837		47452
LOD		3.6		1.9
LOQ		10.9		5.8

Olmesartan medoxomil



Olmesartan medoxomil RS A



Olmesartan medoxomil (USP)

Water Determination <USP 921>

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

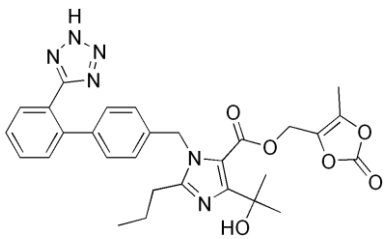
In the case of Olmesartan Medoxomil the water determination can be carried out without problems according to standard methods.

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

Titration one component system

Working Medium: apura® - CombiCoulomat fritless - Coulometric KF reagent for cells with or without diaphragm; 100 mL (1.09257)

Titration parameters
Stirring time: 60s
Default coulometer settings for cell without diaphragm:
For end point indication, e.g.:
I(pol) = 5 - 10 µA, U(EP) = 50 - 100 mV
Stop criterion for fast titration: drift < 20 µg/min
Sample size: 0.4 g (we used Olmesartan medoxomil RS)



Result:
Measured water content in Olmesartan: 0,054% (USP - requirement: < 0,5%)

Procedure
The Karl-Fischer reagent is placed into the titration cell without diaphragm. The coulometer is started and the solvent is titrated dry. After preliminary titration and stabilization of drift the sample is added into the titration cell with a weighing boat (exact sample weight determination by weighing of weighing boat before and after injection) and the water determination is started. For complete dissolution of the sample a stirring time of 60 seconds is recommended.

Product	P/N
CombiCoulomat fritless Karl Fischer reagent for coulometric water determination for cells with and without diaphragm apura®	1.88002

Olmesartan medoxomil (USP)

ICP-MS (USP 232/233)

The sample was tested on a high resolution ICP-MS instrument.
The following metal impurities were measured: Cd, Pb, As, Hg, Ir, Os, Pd, Pt, Rh, Ru, Cu, Mo, Ni, V.

Sample preparation:
0.1 g sample was digested (closed microwave digestion) in 3 mL HNO_3 with 1 mL HCl and 2 mL H_2O_2.

Calibration (using ICP multi-element standards):

For both oral dosage and parenteral dosage the impurities were tested.
Thus, the calibration of the HR-ICP-MS was performed for oral and parenteral dosage.

The limits of impurities are:

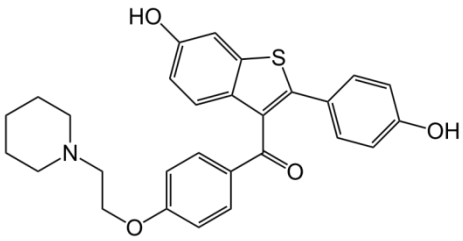
Oral dose			Parenteral dose		
Element		PDE*	Element		PDE*
Iridium	Ir	100	Iridium	Ir	10
Osmium	Os	100	Osmium	Os	10
Palladium	Pd	100	Palladium	Pd	10
Platinum	Pt	100	Platinum	Pt	10
Rhodium	Rh	100	Rhodium	Rh	10
Ruthenium	Ru	100	Ruthenium	Ru	10
Cadmium	Cd	25	Cadmium	Cd	2,5
Lead	Pb	5	Lead	Pb	5
Arsenic	As	1,5	Arsenic	As	1,5
Mercury	Hg	15	Mercury	Hg	1,5
Copper	Cu	1000	Copper	Cu	100
Molybdenum	Mo	100	Molybdenum	Mo	10
Nickel	Ni	500	Nickel	Ni	50
Vanadium	V	100	Vanadium	V	10

*PDE: Permissible Daily Dose based on a person of 50 kg [µg/day]

The calibration standards were diluted in either nitric acid or hydrochloric acid.
For oral dose the ICP multi-element standards (5.05101.0100 and 5.05103.0100) were used.
The multi-element standard (5.05101) that contains Cd, Pb, As, Hg, Cu, Mo, Ni, V was diluted in nitric acid.
The multi-element standard (5.05103) that contains Ir, Os, Pd, Pt, Rh, Ru was diluted in hydrochloric acid.

For parenteral dose the ICP multi-element standards 5.05102.0100 and 5.05104.0100 were used.
The multi-element standard 5.05102 that contains Cd, Pb, As, Hg, Cu, Mo, Ni, V was diluted in nitric acid.
The multi-element standard 5.05104 that contains Ir, Os, Pd, Pt, Rh, Ru was diluted in hydrochloric acid.

Raloxifene Hydrochloride (USP)



Raloxifene is an oral selective estrogen receptor modulator (SERM) that has estrogenic actions on bone and anti-estrogenic actions on the uterus and breast. It is used in the prevention of osteoporosis in postmenopausal women.

Common commercial brand name: Evista
Raloxifene was developed by Eli Lilly and Company.
Sales in 2010 were $1.3 billion globally.
Patent expired in 2014

In this application compilation, we have followed the experimental conditions in USP37-NF32. for raloxifene hydrochloride.

Identification – FTIR (197K)
Assay – HPLC and UHPLC (isocratic methods)
Related Substances (RS) – HPLC (gradient method)

The Assay and Related Substances (RS) have been carried out with HPLC using RP-8 and RP-18 endcapped columns . The assay method has been, in addition, scaled to a shorter UHPLC column dimension with different particle size.

Raloxifene Hydrochloride (USP)

Definition:

Raloxifene Hydrochloride contains NLT 97.5% and NMT 102.0% of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$), calculated on the dried basis.

Identification

A. Infrared Absorption <197K>
B. Identification Tests—General, Chloride191: It meets the requirements, the sample being dissolved in methanol.

Assay: HPLC

Buffer: Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.5 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1.

Mobile phase: Acetonitrile and Buffer (33:67)

System suitability solution: Prepare as directed in the test for Organic Impurities.

Standard solution: 0.05 mg/mL of USP Raloxifene Hydrochloride RS in Mobile phase

Sample solution: 0.05 mg/mL of Raloxifene Hydrochloride in Mobile phase

Chromatographic system (See Chromatography 621, System Suitability.)

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 3.5 µm base-deactivated packing L7

Column temperature: 35°C

Flow rate: 1.5 mL/min *(we used 1.0 mL/min for HPLC method and 0.21 mL/min for UHPLC method)*

Injection volume: 10 µL

Raloxifene Hydrochloride (USP)

System suitability
Sample: System suitability solution

Suitability requirements
Resolution: NLT 2.0 between raloxifene and raloxifene related compound C
Tailing factor: NMT 2.0 for raloxifene
Relative standard deviation: NMT 0.7% for raloxifene

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of raloxifene hydrochloride ($C_{28}H_{27}NO_4S \cdot HCl$) in the portion of Raloxifene Hydrochloride taken:

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

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

Acceptance criteria: 97.5%–102.0% on the dried basis

IMPURITIES - Organic Impurities — HPLC

Solution A: Dissolve 9.0 g of monobasic potassium phosphate in 1000 mL of water. Add 0.6 mL of phosphoric acid, and adjust with phosphoric acid or potassium hydroxide solution to a pH of 3.0 ± 0.1.
Solution B: Acetonitrile
Mobile phase: See Table 1.
[Note—Adjust the start time of the gradient step on the basis of the instrument's dwell volume.]

Time (min)	Solution A (%)	Solution B (%)
0.00	75	25
9.00	75	25
40.25	50	50
42.25	75	25
49.00	75	25

Raloxifene Hydrochloride (USP)

Diluent A: Solution A and acetonitrile (70:30)
Diluent B: Tetrahydrofuran and methanol (70:30)

Raloxifene related compound C solution: 0.15 mg/mL of USP Raloxifene RS C in Diluent B
System suitability solution: Transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of Raloxifene related compound C solution, and dilute with Diluent A to volume.
Standard solution: 0.003 mg/mL of USP Raloxifene Hydrochloride RS in Diluent A
Sample solution: 3 mg/mL of Raloxifene Hydrochloride in Diluent A

Chromatographic system (See Chromatography 621, System Suitability.)
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; 5 µm base-deactivated packing L7
Column temperature: 35°C
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: System suitability solution

Suitability requirements
Resolution: NLT 3.0 between raloxifene and raloxifene related compound C
Tailing factor: NMT 2.0 for raloxifene

Analysis
Samples: Standard solution and Sample solution

Record the chromatograms for NLT two times the retention time of the raloxifene peak, and measure all of the peak responses.

Calculate the percentage of each impurity in the portion of Raloxifene Hydrochloride taken:

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

rU = peak response of each impurity in the Sample solution
rS = peak response of raloxifene in the Standard solution
CS = concentration of USP Raloxifene Hydrochloride RS in the Standard solution (mg/mL)
CU = concentration of the Sample solution (mg/mL)

Acceptance criteria: See Table 2. The reporting level for impurities is 0.05%.

Raloxifene Hydrochloride (USP)

Name	RRT	Acceptance criteria (%)
Raloxifene 3,7-diketone[a]	0.74	0.20
Raloxifene	1.00	-
Other impurities	-	0.10
Total impurities	-	0.5

a) Methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3,7-diyl]bis[4-[2-(1-piperidinyl)ethoxy]phenyl].

Observe in USP 38-NF33 it is mentioned to delete the following:
Heavy Metals, Method II231: NMT 10 ppm (Official 1-Dec-2015)

ADDITIONAL REQUIREMENTS
Packaging and Storage: Preserve in tight containers, and store at controlled room temperature.

USP Reference Standards

USP Raloxifene Hydrochloride RS
USP Raloxifene Related Compound C RS
1-(2-{4-[6-Hydroxy-2-(4-hydroxyphenyl)benzothiophene-3-carbonyl]phenoxy}ethyl)piperidine 1-oxide. ($C_{28}H_{27}NO_5S$)

Recommended Merck Millipore products:

FTIR – Identification (197K)
Potassium bromide for IR spectroscopy Uvasol® (1.04907)

HPLC Assay and Related Substances
Purospher® STAR RP-8 endcapped (3 µm) 150x4.6 mm (1.50009.7220) for assay scaled to
Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm (1.50629.0001)
Purospher® STAR RP-8 endcapped (5µm) 250x4.6 mm (1.51454.0001) for RS analysis
Potassium dihydrogen phosphate for analysis (<= 0.005% Na) EMSURE® ACS,ISO,Reag. Ph Eur 104877
Water (LiChrosolv® 1.15333 or water from a Milli-Q system)
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur 100573
Potassium hydroxide solution 47% for analysis EMSURE® 105545
Acetonitrile (isocratic grade for liquid chromatography LiChrosolv®) 1.14291

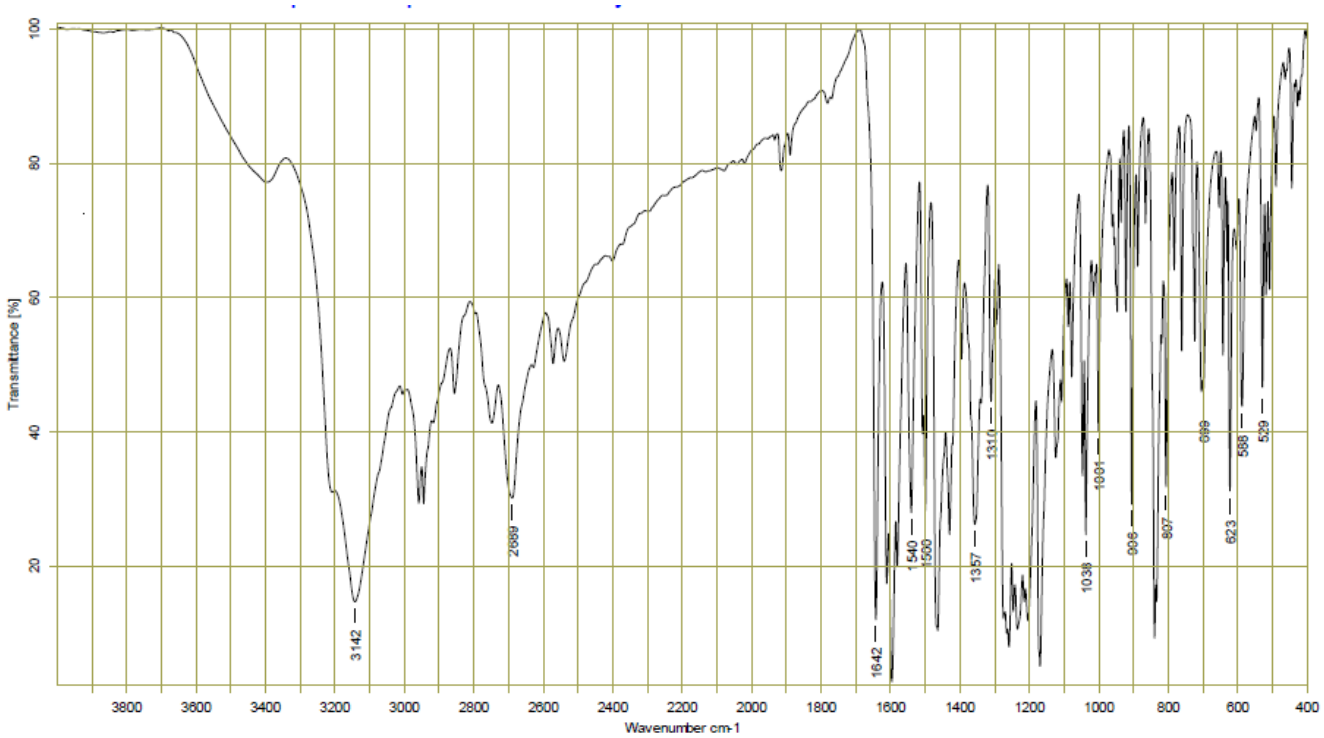
Raloxifene Hydrochloride (USP)

Identification

A. INFRARED ABSORPTION <197K> FTIR

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

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

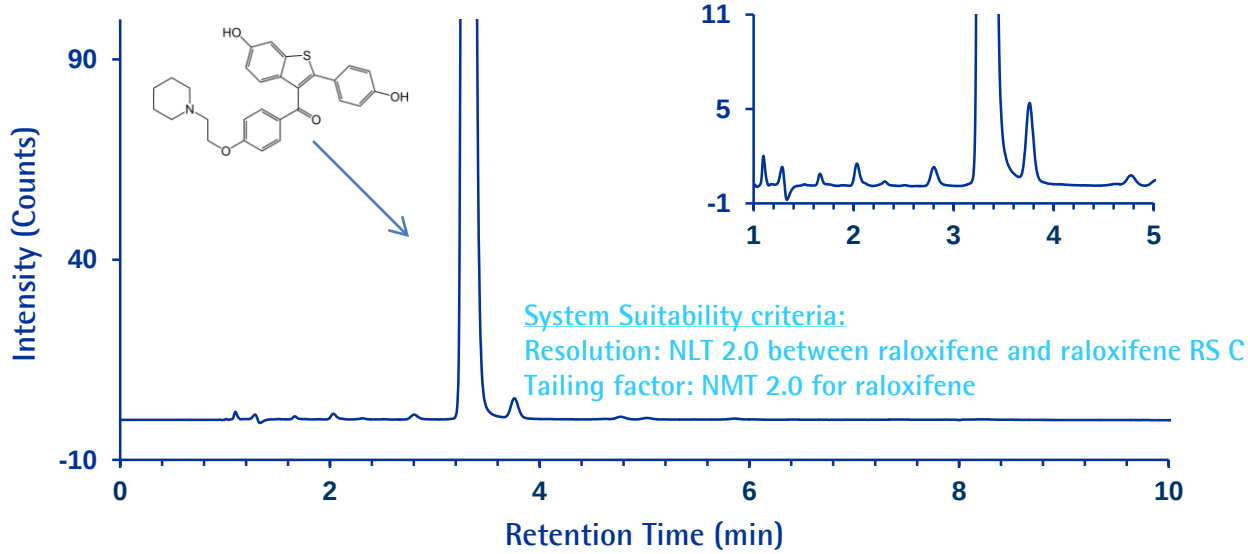


Raloxifene Hydrochloride (USP) – Assay

Purospher® STAR RP-8 endcapped HPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (3 µm) 150x4.6 mm	1.50009.7220
Injection:	10 µL	
Detection:	UV 280nm	
Cell:	11 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.3 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1. Mix acetonitrile and buffer (33:67 v/v)	
Temperature:	35°C	
Diluent:	Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.	
Samples:	**Standard solution:** 0.05 mg/mL of USP Raloxifene Hydrochloride RS in Mobile phase **Sample solution:** 0.05 mg/mL of Raloxifene Hydrochloride in Mobile phase **System suitability solution:** transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of Raloxifene related compound C solution, and dilute with Diluent A to volume.	
Pressure Drop:	172 Bar (2494psi)	



System Suitability Solution

System Suitability criteria:
Resolution: NLT 2.0 between raloxifene and raloxifene RS C
Tailing factor: NMT 2.0 for raloxifene

Chromatographic Data: (SST)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	1.3			
Raloxifene RS	3.3		9337	1.14
Raloxifene RS C	3.8	3.4	11166	1.25

Raloxifene Hydrochloride (USP) - Assay

Validation and Verification HPLC - Assay

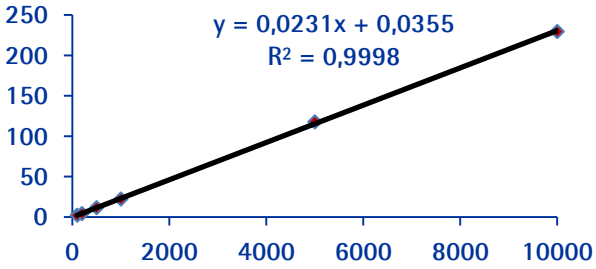
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Raloxifene HCl and Raloxifene RS C using a Purospher® STAR RP-8 endcapped (3 µm) 150x4.6 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Raloxifene HCl	3.34	-	1.16	-
Raloxifene RS C	3.95	0.85	1.05	3.4

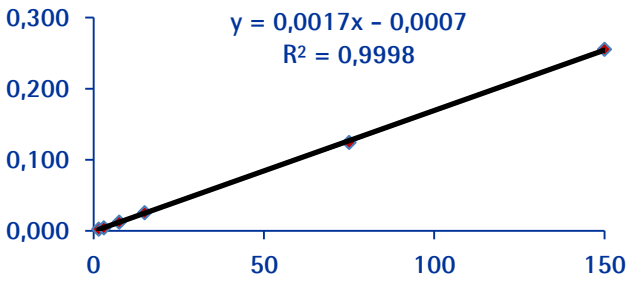
2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 100-10000 ppm of Raloxifene HCl, and six (6) concentration levels ranging from 1.5-150 ppm of Raloxifene RS C.

	[Raloxifene] (ppm)	Area (mAU*min)	[Raloxifene RS C] (ppm)	Area (mAU*min)
	100	2.378	1.5	0.002
	200	4.305	3.0	0.004
	500	11.498	7.5	0.012
	1000	22.221	15	0.026
	5000	117.685	75	0.124
	10000	229.634	150	0.255
STEYX		0.611		0.000662
SLOPE		0.0236		0.00166
LOD		85		1.3
LOQ		259		4.0

Raloxifene



Raloxifene RS C

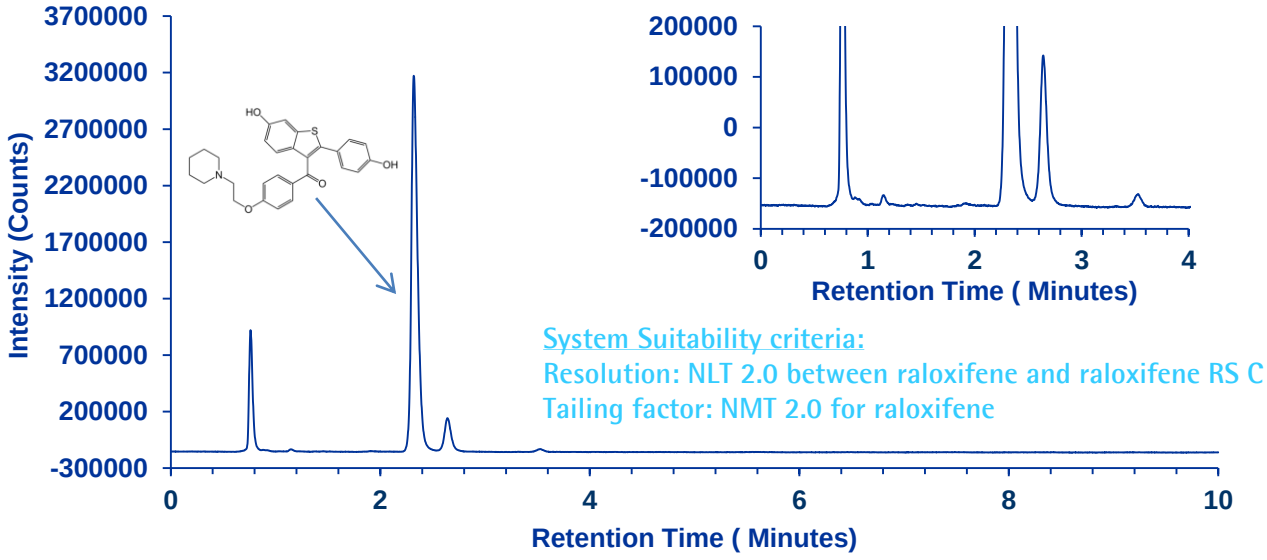


Raloxifene Hydrochloride (USP) – Assay

Purospher® STAR RP-8 endcapped UHPLC

Chromatographic Conditions

Column:	Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm	1.50629.0001
Injection:	2 µL	
Detection:	UV 280nm	
Cell:	1.4 µL	
Flow Rate:	0.21 mL/min	
Mobile Phase:	Dissolve 7.2 g of monobasic potassium phosphate in 1000 mL of water. Add 1.3 mL of phosphoric acid, and further adjust with phosphoric acid or potassium hydroxide solution to a pH of 2.5 ± 0.1. Mix acetonitrile and buffer (33:67 v/v)	
Temperature:	35°C	
Diluent:	Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.	
Samples:	**Standard solution:** 0.05 mg/mL of USP Raloxifene Hydrochloride RS in Mobile phase **Sample solution:** 0.05 mg/mL of Raloxifene Hydrochloride in Mobile phase **System suitability solution:** Transfer 15 mg of USP Raloxifene Hydrochloride RS to a 50-mL volumetric flask, add 1.0 mL of Raloxifene related compound C solution, and dilute with Diluent A to volume.	
Pressure Drop:	261 Bar (3785psi)	



System Suitability Solution

System Suitability criteria:
Resolution: NLT 2.0 between raloxifene and raloxifene RS C
Tailing factor: NMT 2.0 for raloxifene

Chromatographic Data: (SST)

Compound	Retention Time (min)	Resolution	Plates	Tailing Factor
t0 void volume	0.9			
Raloxifene RS	2.3		7051	1.13
Raloxifene RS C	2.6	2.8	8119	1.11

Raloxifene Hydrochloride (USP) - Assay

Validation and Verification UHPLC - Assay

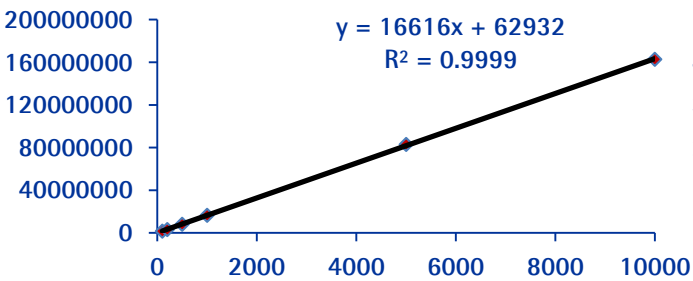
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Raloxifene HCl and Raloxifene RS C using a Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Raloxifene HCl	2.32	-	1.3	-
Raloxifene RS C	2.64	0.88	1.1	2.8

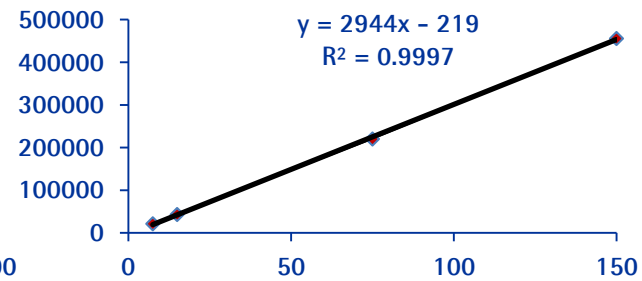
2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 100-10000 ppm of Raloxifene HCl, and four (4) concentration levels ranging from 7.5-150 ppm of Raloxifene RS C.

	[Raloxifene] (ppm)	Area (counts)	[Raloxifene RS C] (ppm)	Area (counts)
	100	1548778	7.5	21459
	200	3093681	15	43233
	500	8091485	75	220077
	1000	16499725	150	455731
	5000	82905592		
	10000	162787912		
STEYX		62932		219
SLOPE		16616		2944
LOD		12.5		0.25
LOQ		38		0.75

Raloxifene



Raloxifene RS C

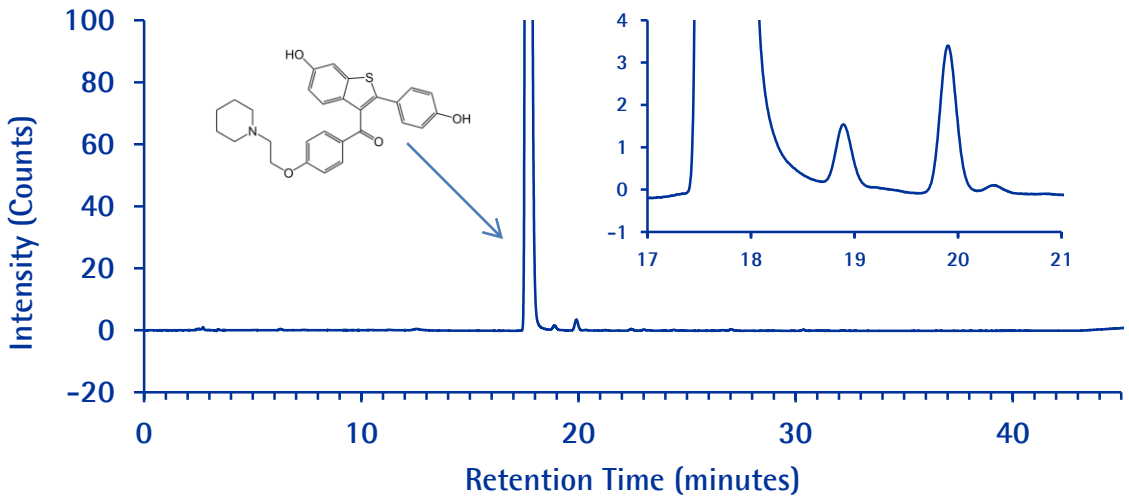


Raloxifene HCl (USP) – Related Substances

Purospher® STAR RP-8 endcapped HPLC

Column:	Purospher® STAR RP-8 endcapped (5µm) 250x4.6 mm	1.51454.0001
Injection:	10 µL	
Detection:	UV 280nm	
Cell:	11 µL	
Flow Rate:	1.5mL/min	
Solution A:	Assay solution A : Acetonitrile 75:25	
Solution B:	Assay solution A : Acetonitrile 50:50	
Gradient:	See table	
Temperature:	35°C	
Diluent	Acetonitrile:Buffer 60:40	
SST stock solution:	Transfer 15mg of USP Raloxifene Hydrochloride RS to a 50 mL volumetric flask and add 15mL Acetonitrile, 3mL water and 5mL of 30% hydrogen peroxide. Shake the solution for 30min, followed by 30min sonication. Let it stand at least for 6h at 30°C. Dilute with diluent 1 to 50mL	
SST solution:	15mg Raloxifene HCl to a 50mL volumetric flask, add 5mL of system suitability stock solution and 20mL of Diluent 2. Dilute with impurity solution A.	
Pressure Drop:	81-145Bar (1175-2103psi)	

Time (min)	Solution A (%)	Solution B (%)
0.00	75	25
9.00	75	25
40.25	50	50
42.25	75	25
49.00	75	25



System Suitability criteria:
Resolution: NLT 3.0 between raloxifene and raloxifene RS C
Tailing factor: NMT 2.0 for raloxifene

Chromatographic Data: (SST)

Compound	Retention Time (min)	RRT	Resolution	Plates	Tailing Factor
t0 void volume	3.4	-			
Raloxifene RS	17.3	0.86		66831	1.10
Raloxifene RS C	19.9	1.0	6.1	72260	1.06

Raloxifene HCl (USP) – Related Substances

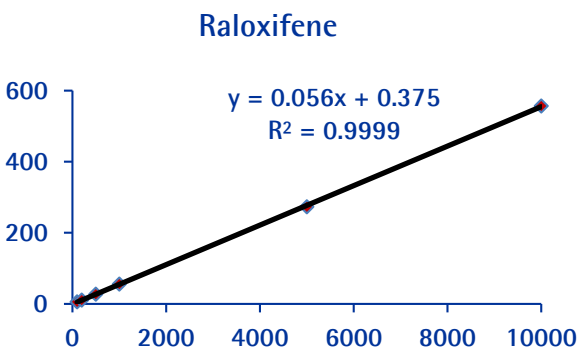
Validation and Verification HPLC

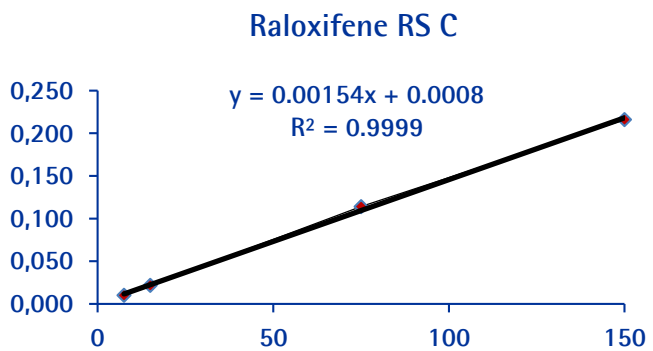
1. **Specificity**
   Determined by injection of SST Solution and determination of the retention time and relative retention time for Raloxifene HCl and Raloxifene RS C using a Purospher® STAR RP-8 endcapped (5 µm) 250x4.6 mm column.

Compound	Retention Time (min)	RRT	Tailing factor	Resolution
Raloxifene HCl	17.3	0.89	1.09	-
Raloxifene RS C	19.9	1.0	1.12	6.09

2. **Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).**
   Determined by injecting six (6) concentration levels from 100-10000 ppm of Raloxifene HCl, and six (6) concentration levels ranging from 1.5-150 ppm of Raloxifene RS C.

	[Raloxifene] (ppm)	Area (mAU*min)	[Raloxifene RS C] (ppm)	Area (mAU*min)
	100	5.599	1.5	0.010
	200	10.812	3.0	0.022
	500	27.328	7.5	0.114
	1000	55.919	15	0.216
	5000	273.231	75	0.010
	10000	556.720	150	0.022
STEYX		0.375		8.3E-05
SLOPE		0.056		0.00154
LOD		22		0.18
LOQ		67		0.54





Solvents and Reagents

Product	P/N
Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00063
Formic acid 98-100% for analysis EMSURE® ACS,Reag. Ph Eur	1.00264
Hydrochloric acid (fuming 37% for analysis EMSURE® ACS,ISO,Reag. Ph Eur)	1.00317
Hydrochloric Acid, 30% Ultrapur	1.01514
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1.00573
Trifluoroacetic acid for spectroscopy Uvasol®	1.08262
Potassium hydroxide solution 47% for analysis EMSURE®	1.05545
Sodium hydroxide solution 50% for analysis EMSURE®	1.58793
Sodium dihydrogen phosphate dihydrate for analysis EMSURE® Reag. Ph Eur	1.06342
di-Sodium hydrogen phosphate dihydrate for analysis EMSURE®	1.06580
tri-Sodium phosphate dodecahydrate for analysis EMSURE® ACS,Reag. Ph Eur	1.06578
Potassium dihydrogen phosphate for analysis (<= 0.005% Na) EMSURE® ACS,ISO,Reag. Ph Eur	1.04877
Acetonitrile hypergrade for LC-MS LiChrosolv®	1.00029
Acetonitrile (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur	1.00030
Acetonitrile (isocratic grade for liquid chromatography) LiChrosolv®	1.14291
Methanol (gradient grade for liquid chromatography) LiChrosolv® Reag. Ph Eur	1.06007
Water for chromatography (or use a Milli-Q Integral Water Purification System)	1.15333
Chromolith® HighResolution RP-18 endcapped 100x4.6 mm	1.52022
Purospher® STAR RP-18 endcapped (5 µm) 150x4.6 mm	1.51455
Purospher® STAR RP-18 endcapped (3 µm) 100x4.6 mm	1.50469
Purospher® STAR RP-18 endcapped (3 µm) 100x2.1mm	1.50653
Purospher® STAR RP-18 endcapped (2 µm) 50x2.1mm	1.50651
Purospher® STAR RP-8 endcapped (5 µm) 250x4.6 mm	1.51454
Purospher® STAR RP-8 endcapped (5 µm) 150x4.6 mm	1.51453
Purospher® STAR RP-8 endcapped (3 µm) 150x4.6 mm (NOTE – customized packing!)	1.50009.7220
Purospher® STAR RP-8 endcapped (3 µm) 100x4.6 mm (NOTE – customized packing!)	1.50013.7220
Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm	1.50629

Solvents and Reagents

Product	P/N
Karl Fischer	
CombiCoulomat fritless Karl Fischer reagent for coulometric water determination for cells with and without diaphragm apura®	1.88002
CombiCoulomat fritless - Coulometric KF reagent for cells with or without diaphragm; 100 mL	1.09257
CombiTitrant 5 one-component reagent for volumetric KF titration 1 ml = ca. 5 mg H2O apura®	1.88005
CombiSolvent methanol-free for volumetric KF titration with one component reagents apura®	1.88008
ICP	
Hydrochloric acid 30% Suprapur®	1.00318
Hydrogen peroxide 30% Suprapur®	1.07298
Nitric acid 65% Suprapur®	1.00441
Elements: As, Cd, Cu, Hg, Mo, Ni, Pb, V	
ICP Multi-element standard USP-I according to USP <232> oral dose. Certipur®	5.05101
ICP Multi-element standard USP-II according to USP <232> parenteral dose. Certipur®	5.05102
Elements: Ir, Os, Pd, Pt, Rh, Ru	
ICP Multi-element standard USP-III according to USP <232> oral dose 100 mg/l. Certipur®	5.05103
ICP Multi-element standard USP-IV according to USP <232> parenteral dose 10 mg/.Certipur®	5.05104
AAS	
Lanthanum(III) oxide for atomic absorption spectroscopy	1.10982
FTIR	
Potassium bromide for for IR spectroscopy Uvasol®	1.04907
Dissolution	
Millex PTFE filter	

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