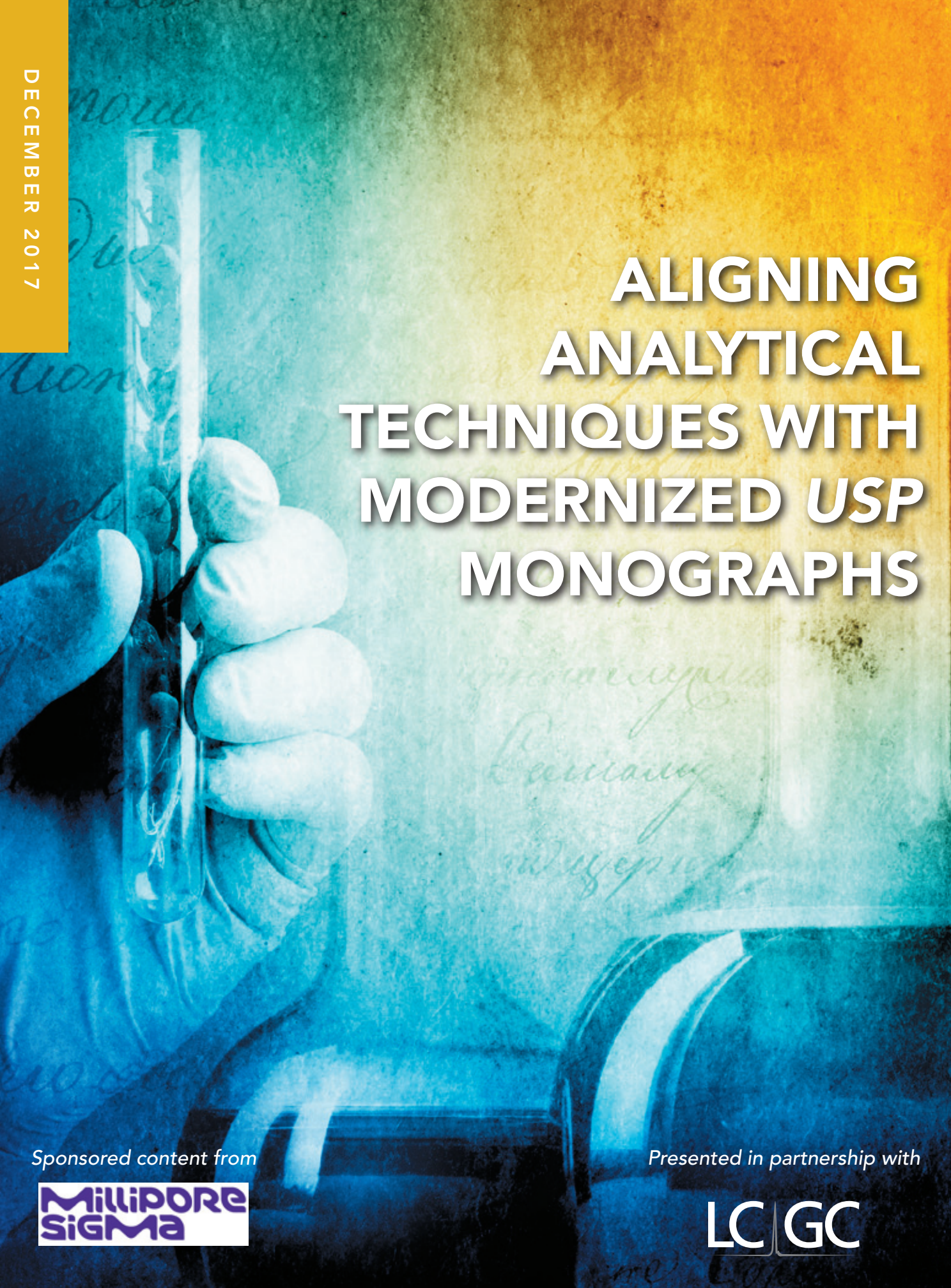
DECEMBER 2017

# ALIGNING ANALYTICAL TECHNIQUES WITH MODERNIZED *USP* MONOGRAPHS



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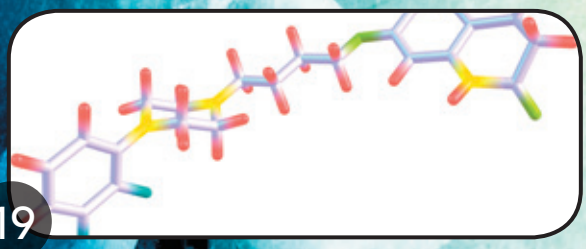






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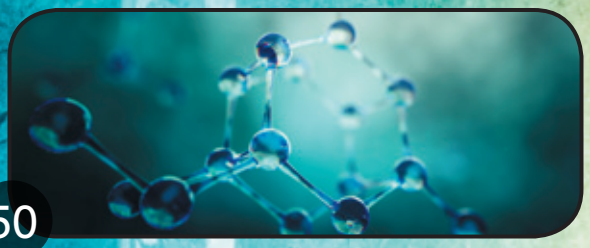



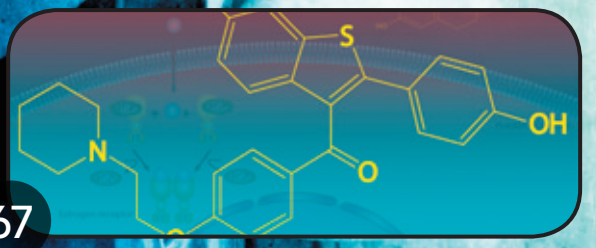




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# SOLUTIONS FOR REGULATED PHARMACEUTICAL INSTRUMENTAL ANALYSIS

**In this section:**



## Overview

### Background Information

This compilation of monographs presents complete solutions for regulated pharmaceutical instrumental analysis with a focus on the testing of prescription and over-the-counter small-molecule drugs. All highlighted methods (18 in total) are compliant with requirements in the USP 40–NF 35. We use our high-quality products in all examples, which you may use for validated pharmaceutical quality control.

A monograph represents a published standard method by which the use of one or more substances is authorized. A manufacturer can prove the safety of their products by following the specific method(s) and complying with the specifications; however, the use of a monograph

does not guarantee automatic approval of the drug.

The USP–NF comprises the United States Pharmacopeia (USP) and the National Formulary (NF), and it issues a set of public pharmacopeial standards. The USP supplies monographs for drug substances, dosage forms, compounded preparations, dietary supplements, and ingredients; the NF supplies monographs for excipients.

The Food and Drug Administration (FDA) defines generic drugs as "identical—or bioequivalent—to a brand name drug in dosage form, safety, strength, route of administration, quality, performance characteristics, and intended use."[1] Generic and brand name 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 its pharmacokinetic and pharmacodynamic properties.

In this compilation, you will find high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC) methods for active pharmaceutical ingredient (API) assays, their related substances (impurity profiling), and 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 Fourier transform infrared spectroscopy (FTIR) analysis for identification purposes. We have developed a new liquid chromatography–mass spectrometry (LC-MS) procedure for impurity profiling of olmesartan medoxomil (though this is not an active USP method). All methods are compliant with the system suitability criteria of each corresponding monograph.

## USP General Chapters

The United States Pharmacopeia–National Formulary (USP–NF) guidelines are continuously revised, and the revisions are presented in twice-yearly supplements as standard revisions in the USP–NF. More frequently, the revisions are published through various accelerated revision processes: Errata, interim revision announcements (IRAs), revision bulletins, and Stage 6 Harmonization. These notices are posted on the USP website in the USP–NF section.

The monographs highlighted in this compilation are compliant with USP 40–NF 35. The USP general chapters provide details about the tests and procedures referenced by the monographs; the general notices provide definitions for terms used in the monographs and information that is necessary to interpret the monograph requirements.

The following pages show some of the details that are relevant for the analytical techniques used within this compilation, namely USP **<197>** (identification with

IR or FTIR), **<232-233>** (heavy metal analysis with AAS or ICP), **<621>** (chromatography), **<711>** (dissolution) and **<921>** (water determination/KF titration).

## Reference Standards

*"Reference Standards provided by the United States Pharmacopeial Convention (USP Reference Standards, or RS) are highly characterized specimens reflective of specified drugs and foods (drug substances, biologics, excipients, dietary supplements, food ingredients, impurities, degradation products, reagents, and performance verification standards). When appr oved 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 RS are never intended for therapeutic use. USP's RS are provided for legal metrology purposes and can help ensure comparability of results and traceability to Système International d'Unités (SI) units whether certified or not. USP RS are Reference Materials as defined in the International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM): 3rd Edition 2007."*

More information can be found in General Chapters: <11> USP Reference Standards.

## USP Monograph Modernization

The USP began a global initiative to modernize many of the existing monographs and is actively seeking industry collaborators to assist in the development of such monographs.

In order to strengthen the protection of public health, members of the pharmaceutical industry and other interested stakeholders are encouraged to assist in providing updated public standards. The USP intends to modernize monographs as soon as possible, either by traditional submission from a stakeholder or from the USP's 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 following test procedures apply to substances that absorb ultraviolet (UV) or infrared (IR) radiation. "The IR absorption spectrum of a substance, compared with that obtained concomitantly 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."[2]

Seven methods are indicated for the preparation of previously dried specimens and reference standards for analysis[1]:

- **<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 under examination 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>** 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 (3,800 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.[2]

## USP <232> "Elemental Impurities— Limits" and USP <233> "Elemental Impurities–Procedure" – ICP

For more than 100 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 ions, 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—therefore a qualitative test.

We have not carried out this test for any of the aripriprazole, olmesartan medoxomil, or raloxifene monographs, because General Chapter 231 will be replaced in the near future 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 inductively coupled plasma optical emission spectrometry (ICP-OES) and ICP-mass spectrometry (ICP-MS). 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 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q3D Step 2 document as well as other editorial changes. This means that the USP 231 remains official until January 1, 2018, and after this date, this chapter will no longer be required.

Advantages of ICP-based analyses include considerable reductions in sample intake (milligram instead of gram quantities) and the ability to generate quantitative results. General Chapter 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. General Chapter 233 includes information about two reference methods, ICP-OES and 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?
- Can we use a different column dimension?
- Can we scale down to smaller internal diameter columns to save solvent?
- Can we speed up separation?

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

## These factors may affect chromatographic behavior[3]:

- Composition, ionic strength, temperature, and apparent pH of the mobile phase
- Flow rate, column dimensions, column temperature, and pressure
- Stationary phase characteristics, including type of chromatographic support (particle-based or monolithic), particle or macropore size, porosity, and specific surface area
- 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, 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), duration of an initial isocratic hold (when prescribed), or dwell volume is allowed. Additional allowances for gradient adjustment are noted in the following text and tables for USP monographs.

| USP Packings (L classifications) Monolithic HPLC 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 to 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** | $NH_2$ |
| 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 |
| The general description to include **"or a monolithic rod"** to the L8, L10, L11, and L20 packings definition was published in PF 40(6) and implemented in USP 39–NF 34 (2016). | | |

| USP Packings (L classifications) Fused-Core® HPLC Columns | | | |
|---|---|---|---|
| **Packing** | **Description[4]** | **Fused-Core® Silica Particles** | **Chemistry** |
| L1 | Octadecyl silane chemically bonded to porous or non-porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, or a monolithic rod | Ascentis® Express C18 | RP-18, $C_{18}$, ODS |
| L3 | Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic rod | Ascentis® Express HILIC | Silica (Si) |
| L7 | Octylsilane chemically bonded to totally or superficially porous silica particles, 1.5 to 10 µm in diameter, or a monolithic rod | Ascentis® Express C8 | RP-8 $C_8$ |
| L10 | Nitrile groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter, or a monolithic rod | Ascentis® Express ES-Cyano | CN |
| L11 | Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter, or a monolithic rod | Ascentis® Express Phenyl-Hexyl | Phenyl |
| L43 | Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10 µm in diameter | Ascentis® Express F5 | $F_5$ |
| L60 | Spherical, porous silica gel, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped | Ascentis® Express RP-Amide | RP-Amide |
| L86 | Fused core particle with a highly polar ligand possessing 5 hydroxyl groups tethered to the silica gel outer layer, 1.5 to 5 µm in diameter. | Ascentis® Express OH5 | OH |
| [4]USP 40–NF 35 (November 1, 2016) | | | |

# USP Packings (L classifications)

## HPLC Guidelines

### *Particle Size (HPLC)*[6]

For isocratic separations, the particle size and 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 within the range between −25% and +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

| | USP | EP 9 (isocratic methods) |
|---|---|---|
| Column length[5] | 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 (applies to isocratic methods only, no changes allowed for gradients) |
| Flow rate | See separate instructions on next page. **NEW in USP 37** | ±50% |
| Column temperature | ±10 °C | ±10 °C (max 60 °C) |
| Injection volume | This can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits.<br><br>Note that excessive injection volume can lead to unacceptable band broadening, causing a reduction in N and resolution. This applies to both gradient and isocratic separations. | May be decreased (if limit of detection [LOD] and repeatability are OK) |
| pH | ±0.2 units for both isocratic and gradient separations | ±0.2 units (±1.0% for neutral substances) |
| 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 ±2% absolute, whichever is larger |

[5]A guard column may be used with the following requirements, unless otherwise indicated in the individual monograph (USP):
- the length of the guard column must be not more than 15% of the length of the analytical column,
- the inner diameter must be the same or smaller than that of the analytical column, and
- the packing material should be the same as the analytical column (e.g., silica) and contain the same bonded phase.

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

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 used when the adjustment results in a higher number of theoretical plates that generate smaller peak volumes, which may require adjustments to minimize extra-column band broadening by factors such as instrument plumbing, detector cell volume and sampling rate, and injection volume.

For gradient separations, changes in length, column inner diameter, and particle size are not allowed.

### *Flow Rate (HPLC)*[7]

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

| Length (L, mm) | Column diameter (dc, mm) | Particle size (dp, µm) | Relative values | | | | |
|---|---|---|---|---|---|---|---|
| | | | L/dp | F | N | Pressure | Run time |
| 250 | 4.6 | 10 | 25,000 | 0.5 | 0.8 | 0.2 | 3.3 |
| 150 | 4.6 | 5 | 30,000 | 1.0 | 1.0 | 1.0 | 1.0 |
| 150 | 2.1 | 5 | 30,000 | 0.2 | 1.0 | 1.0 | 1.0 |
| 100 | 4.6 | 3.5 | 28,600 | 1.4 | 1.0 | 1.9 | 0.5 |
| 100 | 2.1 | 3.5 | 28,600 | 0.3 | 1.0 | 1.9 | 0.5 |
| 75 | 4.6 | 2.5 | 30,000 | 2.0 | 1.0 | 4.0 | 0.3 |
| 75 | 2.1 | 2.5 | 30,000 | 0.4 | 1.0 | 4.0 | 0.3 |
| 50 | 4.6 | 1.7 | 29,400 | 2.9 | 1.0 | 8.5 | 0.1 |
| 50 | 2.1 | 1.7 | 29,400 | 0.6 | 1.0 | 8.5 | 0.1 |

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%. Additionally, the flow rate can be adjusted by ±50% (isocratic only).

For gradient separations, changes in F, dc, and dp are not allowed.

## Changes in USP 37 - Examples

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

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 × 2.1 mm, 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.[8]

## Injection Volume and Mixture Ratio

### *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 number of theoretical plates and resolution; this applies to both gradient and isocratic separations.[9]

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 the same volume loading on the column. A method scaled from a 250 x 4.6 mm to 100 x 2.1 mm column requires a 12-fold reduction of injection volume using simple volume calculation of a tube (i.e., 250 x 4.6 = 4.15 mL and

100 x 2.1 = 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 the Mobile Phase*[9]

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.

For **binary mixtures** with a specified ratio of 50:50: 30% of 50 is 15% absolute, which 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. For a 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.

For **ternary mixtures** with a specified ratio of 60:35: for the second component, 30% of 35 is 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.

## How to Meet Specifications

### *Wavelength of UV-Visible Detector*[10]

When using a UV-visible detector, a deviation from the specified wavelength is not permitted. 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*

Proper HPLC column choice is a very important consideration; it will be difficult to meet the set requirements in a monograph method with an inappropriate column. In the chapter discussing column selection, we have outlined to which USP classification (code) our HPLC columns belong.

We 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 is important to keep in mind that those columns mentioned in USP as monograph columns are not bound text—the actual monograph only describes the column geometry and classification.

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 and "a listing does not indicate that USP has any particular knowledge of the continued suitability of the product." Listings are compiled based on manufacturer-furnished information, and "the accuracy of the information from these sources cannot be guaranteed." It is recommended that columns be tested with the appropriate sample; data presented in the database should not be relied on to determine USP standards compliance or requirements.

## Validation and Verification

### *System Suitability Test (SST)*

SSTs are used to verify and validate a monograph method and meet the set defined requirements.

1. These tests are used to verify that the chromatographic system is adequate for the intended analysis.[11]
2. The tests are based on the concept that the equipment, electronics, analytical operations, and samples analyzed constitute an integral system that can be evaluated as such.[11]

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 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. The USP therefore developed Chapter 1226 in response to the industry's request to provide instructions for verifying compendial procedures in specific situations.

Here we have addressed the 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. To meet system suitability test (SST) criteria in compendial methods, the role of HPLC columns is of immense importance.

| Validation and Verification | | | | | |
|---|---|---|---|---|---|
| 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 |
| Limit of detection (LOD) | No | Yes | Yes | * | No |
| Limit of quantitation (LOQ) | No | No | No | * | No |
| Linearity | Yes | Yes | No | * | No |
| Range | Yes | Yes | * | * | No |
| *May be required, depending on the nature of the specific test. | | | | | |

### *Validation of Compendial Procedures (General Chapter 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

### *Verification of Compendial Procedures (General Chapter 1226)*

"The intent of this general information chapter is to provide general information on the verification of compendial procedures that are being performed for the first time to yield acceptable results utilizing the personnel, equipment, and reagents available."[12]

Verification consists of assessing selected analytical performance characteristics, such as those described in <1225>, to generate appropriate, relevant data rather than repeating the validation process.[13]

The table below illustrates required tests for the USP chapters that address 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 CFR 211.194 (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. In response to industry inquiries

## USP Chapter 711 – Dissolution

This general chapter is harmonized with the corresponding texts of the European Pharmacopeia or the Japanese Pharmacopeia. These pharmacopeias have undertaken not to make any unilateral change to this harmonized chapter.[14]

This test is provided to determine compliance with the dissolution requirements [where stated in the individual monograph] for dosage forms administered orally. In this general chapter, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified. [Of the types of apparatus described herein, use the one specified in the individual monograph. Where the label states that an article 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 1,000 mL. For media with a pH of 6.8 or greater, pancreatin can be added to produce not more than 1,750 USP units of protease activity per 1,000 mL.][14]

In this compilation, we have tested esomeprazole delayed-release capsules using the following medium: 300 mL of 0.1 N hydrochloric acid. 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.

## USP Chapter 921 – Water Determination

*"Many pharmacopeial articles either are hydrates or contain water in adsorbed form. As a result, the determination of the water content is 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, as directed in the individual monograph. . ."*[15]

The USP has three compendial methods for water determination: <921> Ia (direct titration), Ib (residual titration), and Ic (coulometric titration). The European Union uses mostly method EP 2.5.12, which is equivalent to USP <921> Method Ic. However, determination of content uniformity in Japanese Pharmacopeia General Test 3.05: Water-Solid Interactions, 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 General Chapter 921, we have included one example in this compilation illustrating how to perform volumetric Karl Fischer water determination (Ia)—esomeprazole—and one example with coulometric Karl Fischer water determination (Ic)—olmesartan medoxomil.

## Method Ia – Working Principle

The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.[15]

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 the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the reagent ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy.[15]

## Method Ia – Reagents

***Preparation for the Karl Fischer reagent:*** 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, well shaken, 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.[16]

A commercially available, stabilized solution of Karl Fischer type reagent may be used.[16]

***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 to 250 mg of water.[16]

***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 µA of direct current at about 200 mV of applied potential.[16]

## Method Ic – Working Principle

The Karl Fischer reaction is used in the coulometric determination of water. Iodine, however, 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.[17]

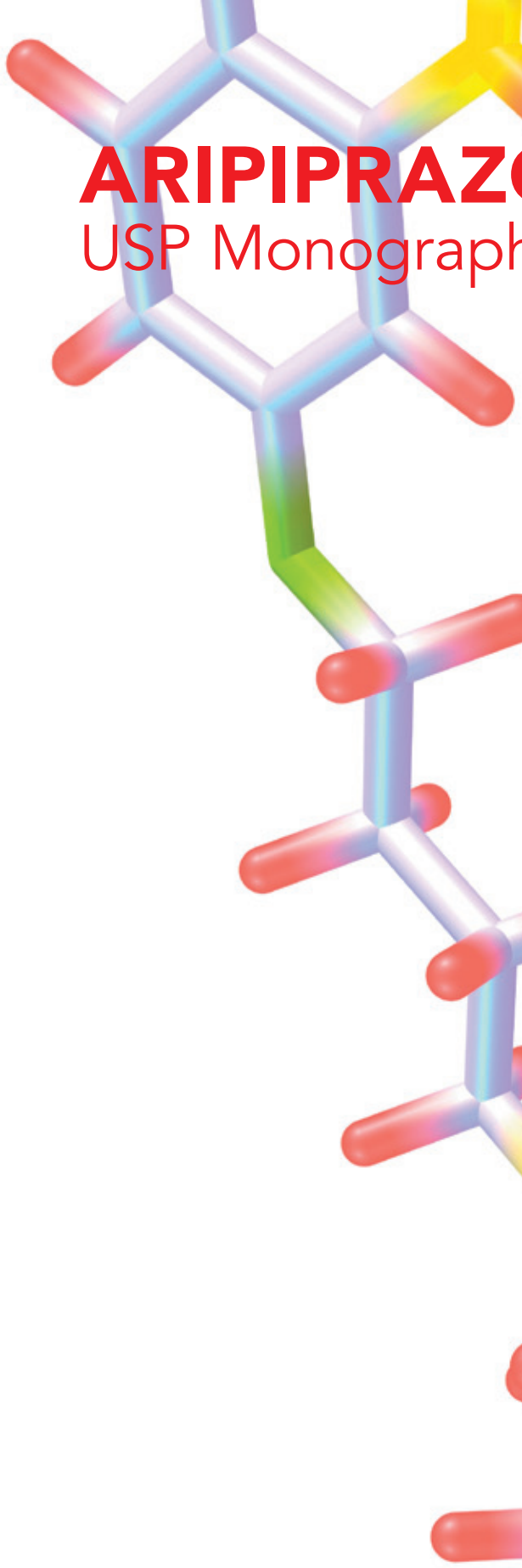
## Method Ic – Reagents

The USP states "see the manufacturer's recommendations." Therefore, we have included a method with recommended procedure in order to proceed with the analysis of olmesartan medoxomil. If you need guidance or suggestions with appropriate reagents, email aquastar@merckgroup.com.

## End Notes

(1) http://www.fda.gov/drugs/resourcesforyou/consumers/buyingusingmedicinesafely/understandinggenericdrugs/ucm144456.htm
(2) Excerpts are from USP 40, General Chapter 197, Spectrophotometric Identification Tests
(3) Excerpt is from USP 40, General Chapter 621, Chromatography.
(4) USP 40–NF 35 (November 1, 2016)
(5) A guard column may be used with the following requirements, unless otherwise indicated in the individual monograph (USP): a)the length of the guard column must be not more than 15% of the length of the analytical column, b)the inner diameter must be the same or smaller than that of the analytical column, and c)the packing material should be the same as the analytical column (e.g., silica) and contain the same bonded phase. In any case, all system suitability requirements specified in the official procedure must be met with the guard column installed.
(6) Implemented and active as of August 1, 2017, from the first supplement (1S) in USP 40.
(7) Excerpt is from USP 40, General Chapter 621, Chromatography.
(8) Table and text are from USP 40, General Chapter 621, Chromatography.
(9) USP 40, General Chapter 621, Chromatography
(10) USP 40, General Chapter 621, Chromatography
(11) Excerpts are from USP 40, General Chapter 621, Chromatography.
(12) Excerpt is from USP 40, General Chapter 1226, Verification of Compendial Procedures. Table is from USP 40, General Chapter 1225, Validation of Compendial Procedures.
(13) Excerpt is from USP 40, General Chapter 1226, Verification of Compendial Procedures.
(14) Excerpts are from USP Stage 6 Harmonization, General Chapter 711, Dissolution (implemented and active as of December 1, 2011). [ ] = USP text: not part of harmonized text.
(15) Excerpts are from USP 40, General Chapter 921, Water Determination.
(16) Excerpts are from USP 40, General Chapter 921, Water Determination.
(17) Excerpt is from USP 40, General Chapter 921, Water Determination.

# ARIPIPRAZOLE
## USP Monograph Methods



**In this section:**



## Overview

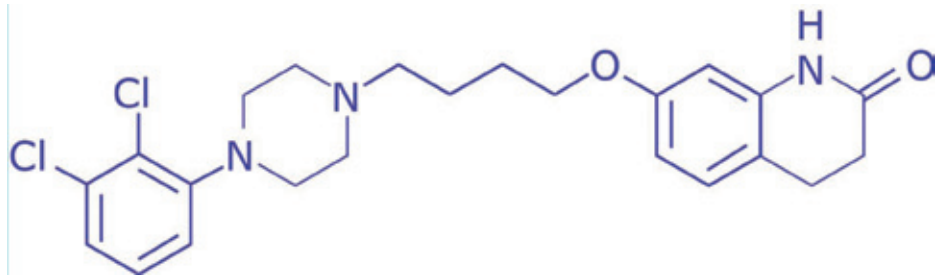
Aripiprazole is an atypical antipsychotic and a partial dopamine agonist. It is primarily used in the treatment of schizophrenia, bipolar disorder, major depressive disorder, tic disorders, and irritability associated with autism. Aripiprazole was first approved by the U.S. Food and Drug Administration in November 2002 for schizophrenia and by 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. In the United States, Otsuka America markets it jointly with Bristol-Myers Squibb. In 2010, sales were $4.6 billion globally; the patent expired in 2015.

In this compilation, we have used the USP 40–NF 35 experimental conditions for aripiprazole in the following areas:




- **Identification**—FTIR
- **Assay**—HPLC (gradient method)
- **Related Substances**—HPLC (gradient method)

The HPLC methods are gradient methods, thus they are nonscalable. The same chromatographic conditions are used for methods in both the assay and related substances, and a full validation protocol can be found using these USP Reference Standards: USP Aripiprazole RS and USP Aripiprazole Related Compound F RS.

## Identification and Assay

### Definition

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

### Identification—FTIR <197K>

- Infrared absorption
- 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 (gradient method)

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 |

*Detector:* UV 254 nm
*Column:* 4.6 mm × 10 cm (3 µm) packing L1
*Flow rate:* 1.2 mL/min
*Injection volume:* 20 µL
*Note: The gradient was established on an HPLC system with a dwell volume of approximately 650 µL.*

We have used a Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Catalogue Number 1.50469).

**This is a gradient method and can therefore not be changed.**

## Assay Solutions and Analysis

***System suitability solution:*** 1 µg/mL each of USP Aripiprazole and USP Aripiprazole Related Compound F in *Diluent*

***Standard solution:*** 0.1 mg/mL of USP Aripiprazole in *Diluent*

***Sample solution:*** 0.1 mg/mL of aripiprazole in *Diluent*

### System Suitability

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

*Note: 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 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 Analysis

***Inorganic Impurities***

- **Residue on Ignition**—USP General Chapter 281: NMT 0.1%
- **Heavy Metals, Method II**—USP General Chapter 231[1]: 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.*

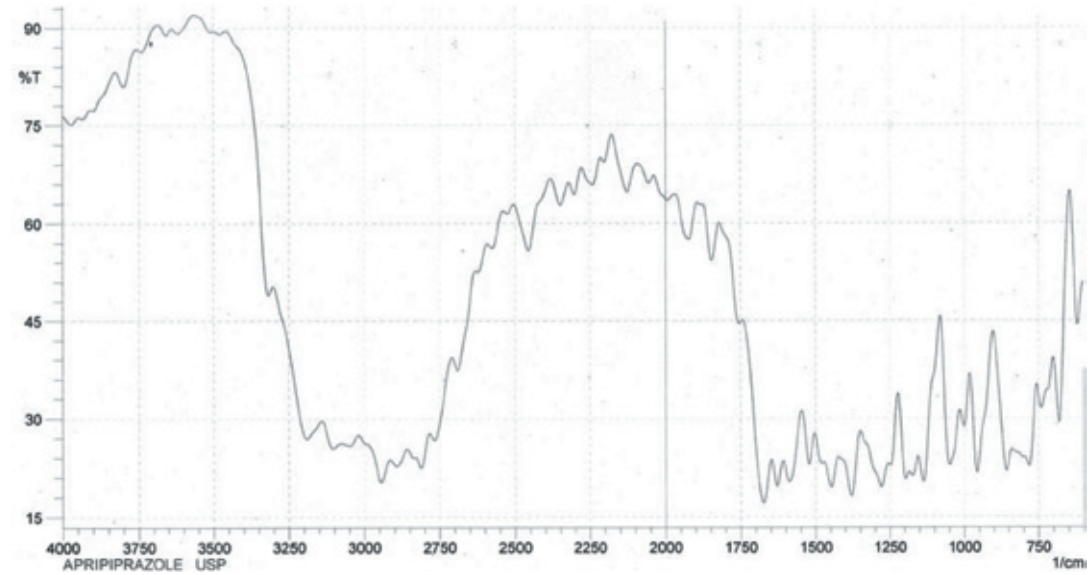
### Analysis

***Samples:*** *Sample solution*

Calculate the percentage of each impurity in the portion of aripiprazole taken.

| Related Substances (Organic Impurities) | | | |
|---|---|---|---|
| Name | Relative retention time (RRT) | Relative response factor (RRF) | Acceptance criteria (NMT, %) |
| Aripiprazole related compound G[1] | 0.9 | 0.72 | 0.10 |
| Aripiprazole | 1.0 | – | – |
| Aripiprazole related compound F[2,3] | 1.1 | 1.0 | – |
| Aripiprazole 4,4-dimer[4] | 1.3 | 1.0 | 0.10 |
| Any other impurity | – | – | 0.10 |
| Total impurities | – | – | 0.50 |

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




***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)

## USP Reference Standards

- USP Aripiprazole

USP Aripiprazole Related Compound F

# Identification Data

## Infrared Absorption

The reference <197K> in a monograph signifies that the substance under

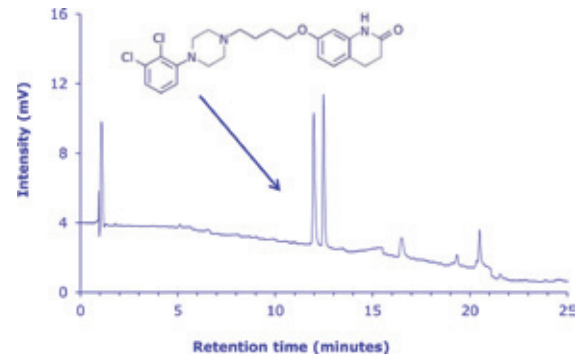
examination is mixed intimately with potassium bromide. We recommend potassium bromide for IR spectroscopy—Uvasol® (Catalogue Number 1.04907).

## Assay and Related Substances Data

### Chromatographic Conditions

| | |
|---|---|
| Column: | Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Catalogue Number 1.50469) |
| 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, and acetic acid (30:10:60:1 v/v) |
| Sample: | 1 µg/mL (1 ppm) each of aripiprazole and aripiprazole related compound F in *Diluent* |
| Pressure drop: | 95–170 Bar (1,377–2,465 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 |




### Suitability requirements

- ***Resolution:***

NLT 2.0 between aripiprazole and aripiprazole related compound F

- ***Tailing factor:***

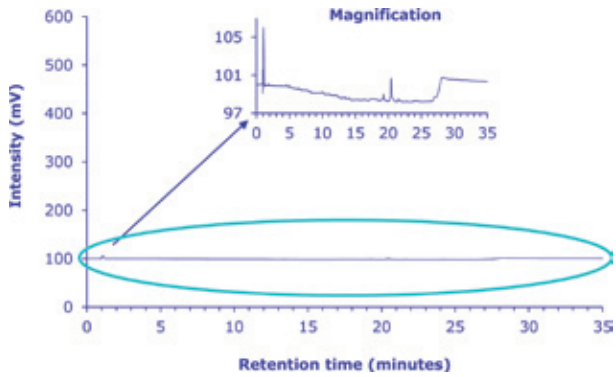
NMT 1.5 for aripiprazole

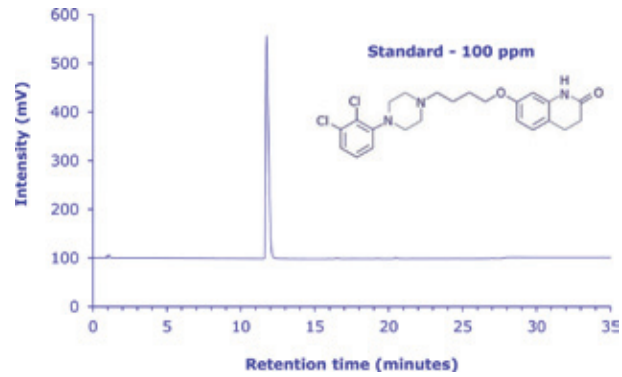
- ***Relative retention time (RRT):***

1.0 for aripiprazole and
1.1 for aripiprazole related compound F

**Chromatographic Data (System Suitability Solution)**

| Compound | Retention time (min) | RRT | Tailing factor | Resolution |
|---|---|---|---|---|
| Aripiprazole | 12.0 | 1.0 | 1.4 | – |
| Aripiprazole related compound F | 12.5 | 1.05 | 1.3 | 2.8 |




| Chromatographic Data (100 ppm Standard solution) | | | |
|---|---|---|---|
| **Compound** | **Retention time (min)** | **Tailing factor** | **Theoretical plates** |
| Aripiprazole | 12.0 | 1.4 | 16,118 |

# Assay and Related Substances Validation Data

## 1. Specificity

Determined by injection of *System suitability solution* and determination of the retention time and relative retention time for aripiprazole and aripiprazole related compound F

| Compound | Retention time (min) | RRT | Tailing factor | Resolution |
|---|---|---|---|---|
| Aripiprazole | 12.0 | 1.0 | 1.4 | – |
| Aripiprazole related compound F | 12.5 | 1.05 | 1.3 | 2.8 |

## 2. Repeatability

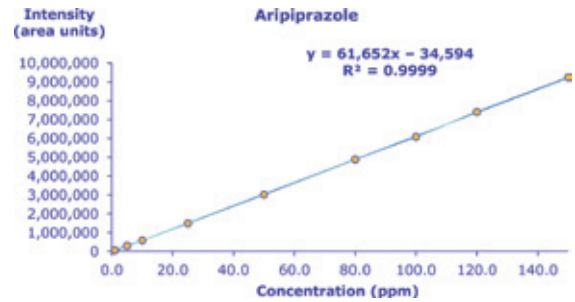
Determined by injecting five samples with a *Standard solution* containing 100.0 ppm USP Aripiprazole and 1.0 ppm of USP Aripiprazole Related Compound F

| Standard solution | Aripiprazole (area units) | Aripiprazole related compound F (area units) |
|---|---|---|
| 1 | 60,114 | 6,047,450 |
| 2 | 60,363 | 6,080,108 |
| 3 | 60,308 | 6,086,256 |
| 4 | 60,174 | 6,081,386 |
| 5 | 60,316 | 6,089,267 |
| Mean | 60,255 | 6,076,893 |
| STDEV | 105.6 | 16,868.6 |
| RSD (%) | 0.18 | 0.28 |

### 3. Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Determined by injecting seven concentration levels from 0.1 to 1.5 ppm of USP Aripiprazole Related Compound F and nine concentration levels ranging from 1.0 to 150.0 ppm of aripiprazole

| Aripiprazole related compound F | | Aripiprazole | |
|---|---|---|---|
| LOD (ppb) | LOQ (ppb) | LOD (ppm) | LOQ (ppm) |
| 7.6 | 23 | 0.57 | 1.7 |

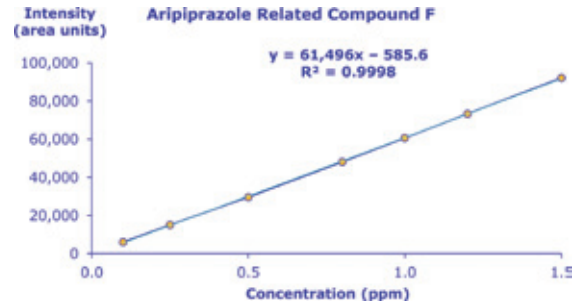



### 4. LOQ Accuracy

Determined by injecting 10 *Standard solutions* at LOQ level of USP Aripiprazole RS

| Injection | Area units | $S_D$ Area units | RSD (%) |
|---|---|---|---|
| N=10 | 151,949 | ±424.9 | 0.28 |

| Aripiprazole related compound F | | Aripiprazole | |
|---|---|---|---|
| (ppm) | Area units | (ppm) | Area units |
| 0.1 | 6,020 | 1.0 | 63,167 |
| 0.25 | 15,062 | 5.0 | 292,127 |
| 0.5 | 29,604 | 10.0 | 586,674 |
| 0.8 | 48,154 | 25.0 | 1,587,549 |
| 1.0 | 60,630 | 50.0 | 3,013,178 |
| 1.2 | 73,335 | 80.0 | 4,878,836 |
| 1.5 | 92,098 | 100.0 | 6,076,893 |
| | | 120.0 | 7,401,807 |
| | | 150.0 | 9,241,987 |



## Recommended Products

- Acetic acid (glacial) 100% anhydrous for analysis—EMSURE® ACS, ISO, Reag. Ph. Eur. (Catalogue Number 1.00063)
- Acetonitrile (gradient grade for liquid chromatography)—LiChrosolv® Reag. Ph. Eur. (Catalogue Number 1.00030)
- Methanol (gradient grade for liquid chromatography)—LiChrosolv® Reag. Ph. Eur. (Catalogue Number 1.06007)
- Potassium bromide for IR spectroscopy—Uvasol® (Catalogue Number 1.04907)
- Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Catalogue Number 1.50469)
- Trifluoroacetic acid for spectroscopy—Uvasol® (Catalogue Number 1.08262)
- Water for chromatography (LC-MS grade)—LiChrosolv® (Catalogue Number 1.15333) or fresh water from the Milli-Q® system
- Aripiprazole United States Pharmacopeia (USP) Reference Standard (Catalogue Number 1042634 USP)
- Aripiprazole Related Compound F United States Pharmacopeia (USP) Reference Standard (Catalogue Number 1042689 USP)

## End Notes

1. Chapter valid until Jan. 1, 2018.

# ESOMEPRAZOLE MAGNESIUM AND ESOMEPRAZOLE MAGNESIUM DELAYED-RELEASE CAPSULES

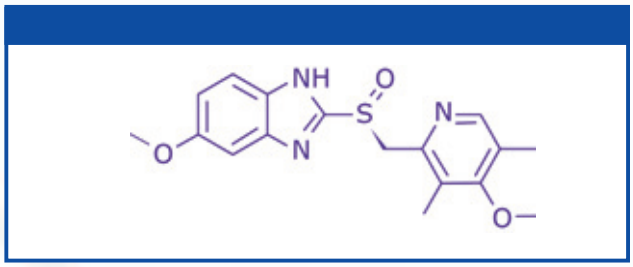
## USP Monograph Methods

**In this section:**

## Overview

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, and Esomezol**

Esomeprazole magnesium was developed by AstraZeneca. In 2010, sales were $4.9 billion globally; the patent expired in 2014.

We have followed the USP 40–NF 35 experimental conditions for esomeprazole magnesium and esomeprazole magnesium delayed-release capsules and applied the following tests:

- **Identification**—FTIR and AAS (content of magnesium)
- **Assay and Related Substances**—HPLC and UHPLC (both isocratic and gradient methods)
- **Water Content**—Karl Fischer Titration
- **Dissolution**—HPLC

This documentation illustrates the complete testing of an active pharmaceutical ingredient (API) and a formulation thereof (capsules), according to the USP test criteria. Identification tests have been carried out with Fourier transformation infrared spectroscopy (FTIR) and atomic absorption spectroscopy (AAS). Assay, related substances (RS), or impurities, 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 conducted in isocratic mode and could therefore be scaled to UHPLC settings relative to the prescribed HPLC column geometry. Because the situation with monolithic columns is similar to that of 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.

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 and reduced column backpressure, and it still meets all method performance criteria.

# Esomeprazole Magnesium USP Monograph Methods: Identification and Assay

## Definition

Esomeprazole magnesium contains not less than (NLT) 98.0% and not more than (NMT) 102.0% of $C_{34}H_{36}MgN_6O_6S_2$, calculated on the anhydrous basis.

## Identification—FTIR <197K> and AAS

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

## Assay—HPLC (gradient method)

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 1,000 mL. Dilute 250 mL of this solution with water to 1,000 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 to a 200-mL volumetric flask, and dissolve in approximately 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 approximately 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 USP General Chapter 621, Chromatography, 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 (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) 150 x 4.6 mm (Catalogue Number 1.51453) for HPLC analysis.

## System Suitability—HPLC

**Sample:** *Standard solution*

## Suitability Requirements

- **Resolution efficiency:** NLT 2,000 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 USP 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 1,000-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:** Use 1,000 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).

**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 minutes. 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—AAS

(See USP General Chapter 851, Spectrophotometry and Light-Scattering.)
*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 of magnesium in µg/mL 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 = concentration of magnesium in the *Sample solution* (µg/mL)
CU = concentration of esomeprazole magnesium in the *Sample solution* (µg/mL)
F = water content of esomeprazole magnesium, as determined in Specific Tests, Water Determination (%)

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

## Impurities Solutions

### Impurities

**Organic Impurities—HPLC (Procedure 1)**

**Solution A:** 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water diluted with water to 1,000 mL. Dilute 250 mL of this solution with water to 1,000 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 each of USP Omeprazole and USP Omeprazole Related Compound A 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 USP General Chapter 621, Chromatography, System Suitability.)

*Detector:* UV 280 nm

*Column:* 125 x 4.0 mm or a 150 x 4.6 mm (5 µm) packing L7

*Note: Alternatively, a 150 x 3.9 mm column (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 the Impurity Table on the below.*

**Impurity Table**

| Name | Relative retention time (RRT) | Acceptance criteria, NMT (%) |
|---|---|---|
| Omeprazole N-oxide[1] | 0.45 | 0.1 |
| Omeprazole sulfone[2] (Omeprazole related compound A) | 0.8 | 0.2 |
| Any other individual impurities | – | 0.1 |
| Omeprazole | 1.0 | – |

[1]4-Methoxy-2-{[(RS)-(5-methoxy-1H-benzimidazol-2yl)sulfinyl] methyl}-3,5-dimethylpyridine 1-oxide
[2]5-Methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl] sulfonyl}-1H-benzo[d]imidazole

## Impurities Analysis and Water Determination

### Suitability Requirements—HPLC

**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 the Impurity Table on the previous page. 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%

### Enantiomeric Purity (Procedure 2):

The recommended column is 100 x 4.6 mm packing L41 (e.g., CHIRALPAK® AGP HPLC Column—100 x 4.6 mm, Catalogue Number 58150AST).

### Water Determination—Karl Fischer

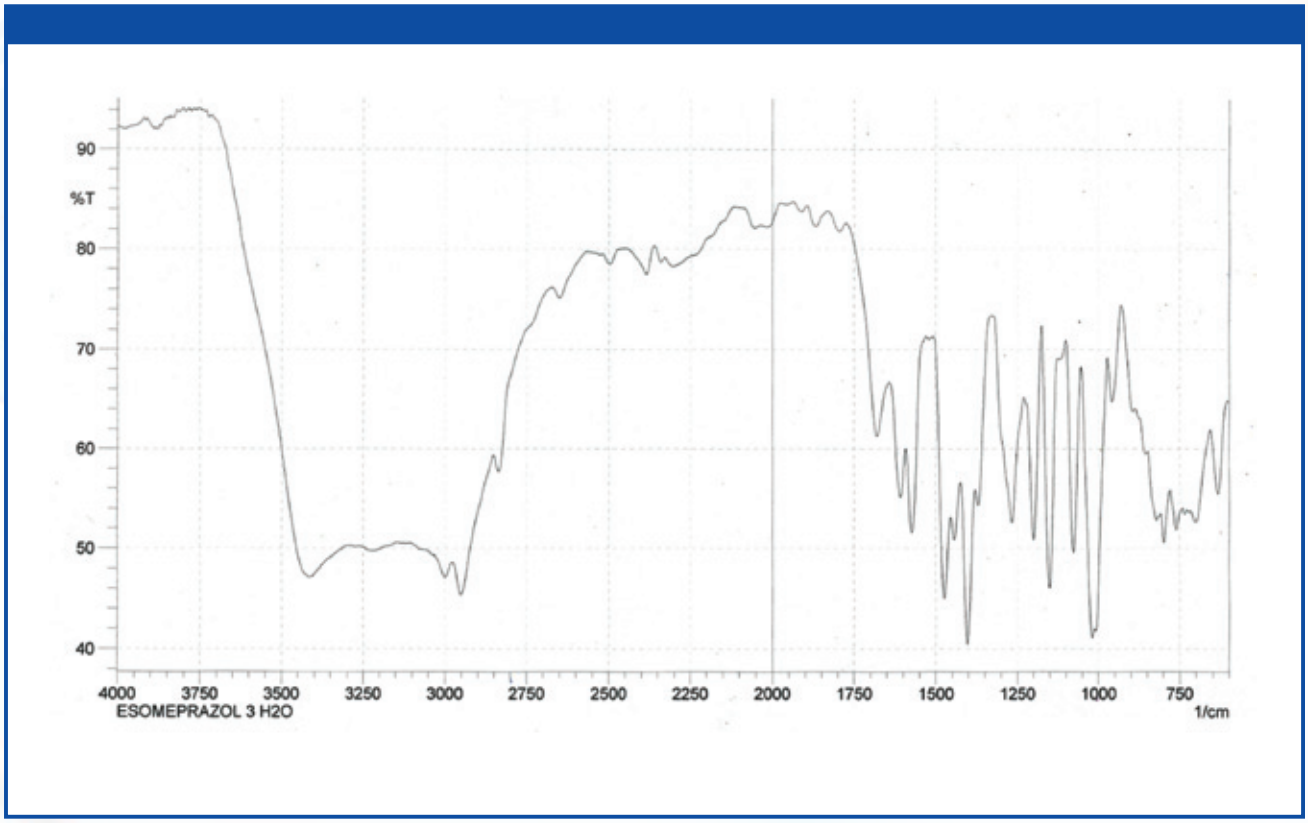
**<921> Method Ia:** 6.0%–8.0%

### Color of Solution

**Sample solution:** Use 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, protect from light, and store at room temperature.

### USP Reference Standards

- USP Esomeprazole Magnesium (Catalogue Number 1249789)
- USP Omeprazole (Catalogue Number 1478505)
- USP Omeprazole Related Compound A (Catalogue Number 1478516) (omeprazole sulfone; 5-methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfonyl}-1H-benzimidazole)

## Identification Data

### A. Infrared Absorption

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

### B. Content of Magnesium

**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 minutes. Transfer 10.0 mL of this solution to a 200 mL volumetric flask,

| | 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 |

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.

**Lanthanum solution:** Transfer 58.7 g of lanthanum oxide into a 1,000-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:** Use 1,000 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 using lanthanum (III) oxide for atomic absorption spectroscopy (Catalogue Number 1.10982), hydrochloric acid 30%—Ultrapur (Catalogue Number 1.01514), and magnesium ICP standard traceable to SRM from NIST $Mg(NO_3)_2$ in HNO3 2–3% 1,000 mg/L Mg—Certipur® (Catalogue Number 1.70331).

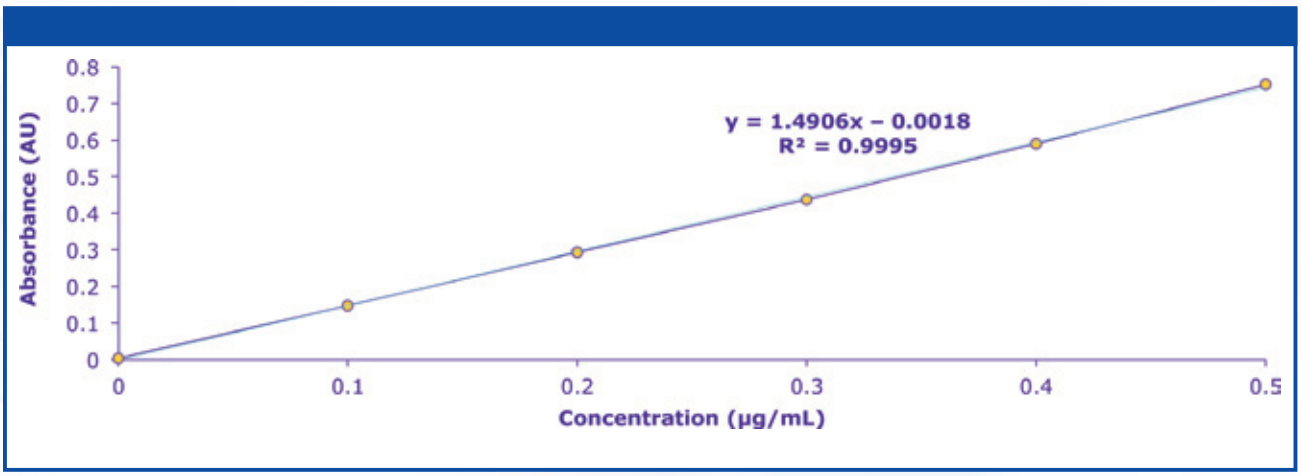
## Identification Data (Content of Magnesium)

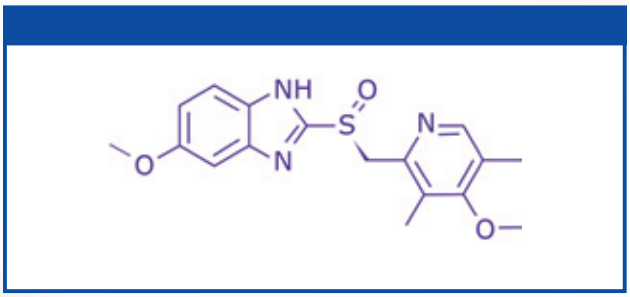
| Identification Data | |
|---|---|
| Concentration (µg/mL) | Absorbance (AU) |
| 0 | 0.003 |
| 0.1 | 0.148 |
| 0.2 | 0.294 |
| 0.3 | 0.437 |
| 0.4 | 0.591 |
| 0.5 | 0.752 |

| Identification Data | |
|---|---|
| Absorbance for sample | 0.563 AU |
| Concentration 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.




## Water Determination <921>

Pharmaceutical products sometimes involve complex formulations.In pharmaceutical guidelines (e.g., USP, Ph. Eur., and DAB), Karl Fischer titration is described as a common method for water determination. However, for certain substances, there are specialized procedures. Difficulties associated with Karl Fischer determination are often caused by limited solubility. In some cases, depending upon the composition and properties of the formulations, it is necessary to consider side reactions. The determination of mass loss as a method of water determination is not recommended.

In the case of esomeprazole, the water determination can be carried out without difficulty using standard methods.

### 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 USP Esomeprazole Magnesium RS.)

**Result:** Measured water content in esomeprazole: 7.63% (USP requirement: 6–8%)

### Procedure

Place the titration medium into the titration cell and titrate dry by means of the titrant. Then add the sample from a weighing boat (for exact sample weight determination,

| Product | Catalogue Number |
|---|---|
| CombiTitrant 5 one-component reagent for volumetric KF titration 1 ml = ca. 5 mg $H_2O$—Apura® | 1.88005 |
| CombiSolvent methanol-free solvent for volumetric KF titration with one component reagents—Apura® | 1.88008 |

weigh the weighing boat before and after the sample addition) and start the titration. For complete dissolution of the sample, we recommend a stirring time of 90 seconds.

## Assay Data

### Chromatographic Conditions

**Column:** Purospher® STAR RP-8 endcapped (5 µm) 150 x 4.6 mm (Catalogue Number 1.51453)

**Injection:** 20 µL

**Detection:** UV 280 nm

**Cell:** 10 µL

**Flow rate:** 1.0 mL/min

**Mobile phase:** Mix acetonitrile and *Buffer* (7:13 v/v).

**Buffer:** 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 1,000 mL. Dilute 250 mL of this solution with water to 1,000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

**Temperature:** 25 °C

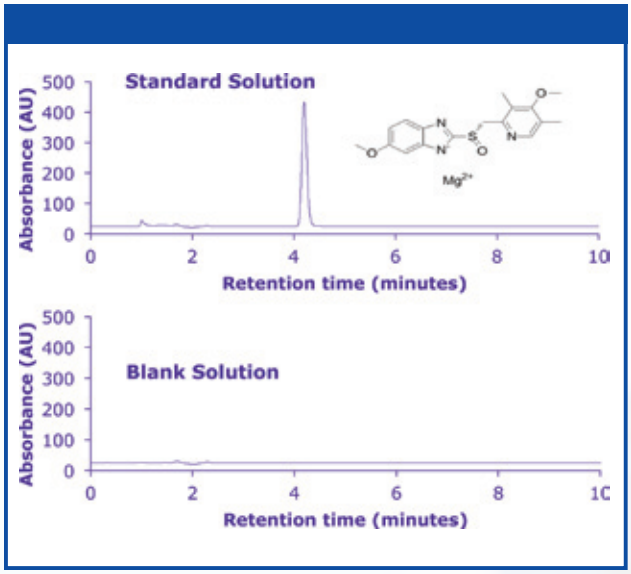
**Diluent:** Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.

**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 diluent, 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 diluent, and dilute with water to final volume.

**Pressure drop:** 101 Bar (1,464 psi)

### Suitability Requirements

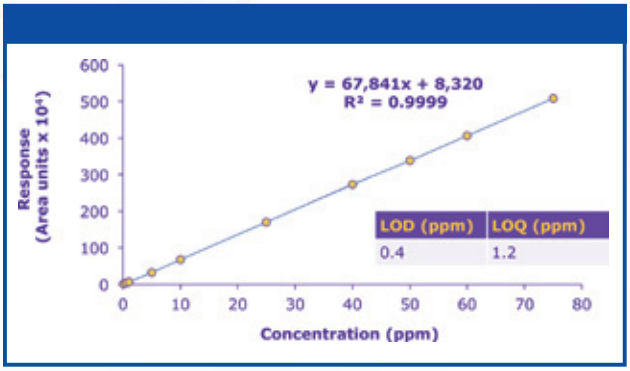



**Column efficiency:** NLT 2,000 theoretical plates

**Suitability Requirements**

| Compound | Retention time (min) | Plates | Tailing factor |
|---|---|---|---|
| Impurity A | – | – | – |
| Omeprazole | 4.2 | 8,269 | 1.1 |

| Linearity | | | |
|---|---|---|---|
| Concentration (ppm) | Area units | Concentration (ppm) | Area units |
| 0.1 | 7,273 | 25 | 1,694,075 |
| 0.5 | 33,723 | 40 | 2,734,520 |
| 1 | 68,763 | 50 | 3,388,150 |
| 5 | 331,460 | 60 | 4,068,780 |
| 10 | 677,630 | 75 | 5,082,225 |




# Related Substances Data (Organic Impurities)

## Chromatographic Conditions 1

**HPLC with a porous, particle-packed column**

**Column:** Purospher® STAR RP-8 endcapped (5 µm) 150 x 4.6 mm (Catalogue Number 1.51453)

**Injection:** 50 µL

**Detection:** UV 280 nm

**Cell:** 10 µL

**Flow rate:** 1.0 mL/min

**Mobile phase:** Mix acetonitrile and *Buffer* (11:29 v/v).

**Buffer:** Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1,000 mL water. If necessary, adjust with phosphoric acid to a pH of 7.6.

**Temperature:** Ambient

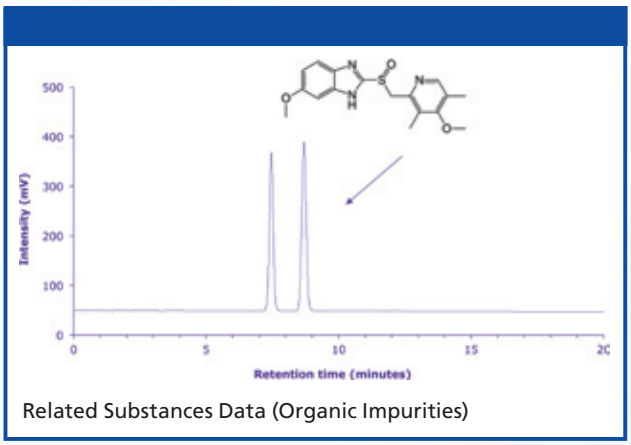
**Diluent:** *Mobile phase*

**System suitability solution:** Dissolve 1.0 mg each of USP Omeprazole and USP Omeprazole Related Compound A in 25 mL of *Diluent*.

**Sample solution:** Dissolve 4.0 mg of sample in 25 mL of *Diluent*.

**Pressure drop:** 87 Bar (1,261 psi)

**Suitability Requirements**



Related Substances Data (Organic Impurities)

- **Resolution:** NLT 3 between omeprazole related compound A and omeprazole
- **Relative retention time (RRT):** 0.8 for omeprazole related compound A and 1.0 for omeprazole

| Chromatographic Data (System Suitability Solution): | | | |
|---|---|---|---|
| Compound | Retention time (min) | Resolution | RRT |
| Omeprazole related compound A | 7.5 | – | 0.85 |
| Omeprazole | 8.7 | 4.2 | 1.00 |

## Chromatographic Conditions 2

**UHPLC with a porous, particle-packed column**

**Column:** Purospher® STAR RP-8 endcapped (2 µm) 100 x 2.1 mm (Catalogue Number 1.50629)

**Injection:** 5 µL

**Detection:** UV 280 nm

**Cell:** 2.5 µL

**Flow rate:** 0.3 mL/min

**Mobile phase:** Acetonitrile and *Buffer* (27:73 v/v)

**Buffer:** Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1,000 mL of water. If necessary, adjust with phosphoric acid to a pH of 7.6.

**Temperature:** Ambient

**Diluent:** *Mobile phase*

**System suitability solution:** Dissolve 1.0 mg each of USP Omeprazole and USP Omeprazole Related Compound A in 25 mL of *Diluent*.

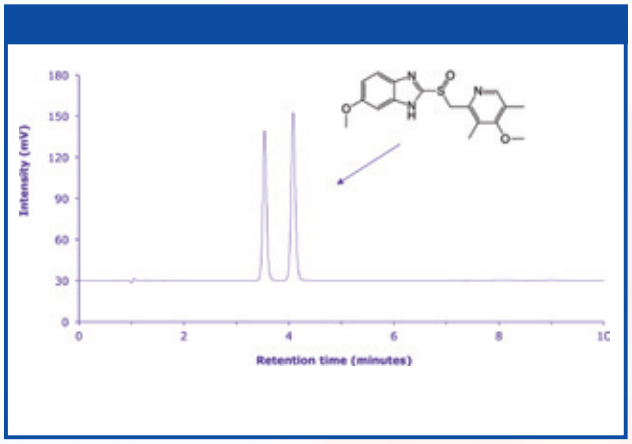
**Sample solution:** Dissolve 4.0 mg of sample in 25 mL of *Diluent*.

**Pressure drop:** 300 Bar (4,350 psi)

Method transfer is possible because of isocratic conditions in the method.

**Alternative column chemistry and geometry:** 150 x 4.6 mm L7 packing

We used a 100 x 2.1 mm L7 packing column (with a scaling factor of 7.2 in volume). We have modified the Mobile phase composition, flow rate, and injection volume within the allowed ranges. The analytical data meet the system suitability requirements.




**Suitability Requirements**

- **Resolution:**
  NLT 3 between omeprazole related compound A and omeprazole

- **Relative retention time (RRT):** 0.8 for omeprazole related compound A and 1.0 for omeprazole

| Chromatographic Data (Sytem Suitability Solution): | | | |
|---|---|---|---|
| Compound | Retention time (min) | Resolution | RRT |
| Omeprazole related compound A | 3.5 | – | 0.85 |
| Omeprazole | 4.1 | 4.2 | 1.00 |

## Chromatographic Conditions 3

**HPLC with a HighResolution monolithic column and alternative packing:**

**Column:** Chromolith® HighResolution RP-18 endcapped 100 x 4.6 mm (Catalogue Number 1.52022)

**Injection:** 20 µL

**Detection:** UV 280 nm

**Cell:** 10 µL

**Flow rate:** 1.0 mL/min

**Mobile phase:** Acetonitrile and *Buffer* (25:75 v/v)

**Buffer:** Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1,000 mL water. If necessary, adjust with phosphoric acid to a pH of 7.6.

**Temperature:** Ambient

**Diluent:** *Mobile phase*

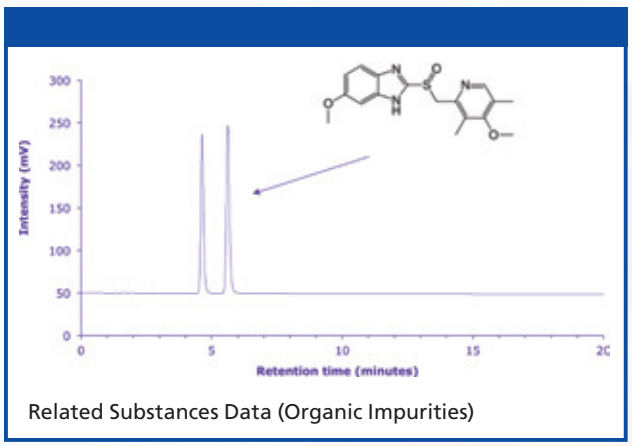
**System suitability solution:** Dissolve 1.0 mg each of USP Omeprazole and USP Omeprazole Related Compound A in 25 mL of *Diluent*.

**Sample solution:** Dissolve 4.0 mg of sample in 25 mL of *Diluent*.

**Pressure drop:** 50 Bar (725 psi)

Method transfer is possible because of isocratic conditions in the method.

**Alternative column chemistry and geometry:** 150 x 3.9 mm (4 µm particle) L1 packing
We used a 150 x 4.6 mm L1 packing column with a monolithic backbone. We have modified the *Mobile phase* composition and reduced the injection volume within the allowed ranges. The acquired analytical data meet the system suitability requirements.



Related Substances Data (Organic Impurities)

**Suitability Requirements**

- **Resolution:** NLT 3 between omeprazole related compound A and omeprazole

- **Relative retention time (RRT):** 0.8 for omeprazole related compound A and 1.0 for omeprazole

| Chromatographic Data (System Suitability Solution): | | | |
|---|---|---|---|
| Compound | Retention time (min) | Resolution | RRT |
| Omeprazole related compound A | 4.6 | – | 0.82 |
| Omeprazole | 5.6 | 4.7 | 1.00 |

# Esomeprazole Delayed-Release Capsules USP Monograph Methods: Identification and Assay

## Identification

Enantiomeric purity (not performed, as it requires a chiral column [100 x 4.0 mm (5 µm) packing L41] that was not available at the time of testing)

## Assay—HPLC

**Buffer:** Prepare a phosphate buffer with a pH of 7.3 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 dilute with water to 1,000 mL.

**Diluent:** Prepare as directed in Identification Test A.

**Mobile phase:** Mix 350 mL of acetonitrile and 500 mL of *Buffer*. Dilute with water to 1,000 mL.

**Standard solution:** Transfer 10 mg of USP Omeprazole 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.

**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:** Dissolve 0.04 mg/mL of esomeprazole from the *Sample stock solution* in water. Protect from light.

## Chromatographic System

(See USP General Chapter 621, Chromatography, System Suitability.)
*Detector:* UV 302 nm
*Column:* 4.6 mm × 15 cm (5 µm) packing L1
*Flow rate:* 1 mL/min
*Injection volume:* 20 µL

## System Suitability

**Sample:** *Standard solution*

# Assay and Dissolution Testing

## 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 in the *Standard solution* (mg/mL)
CU = nominal concentration of esomeprazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### Dissolution—HPLC <711>

***Test 1***

**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 in alcohol. Dilute this solution with pH 6.8 phosphate buffer to obtain a solution containing (L/1,000) 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.

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

## Dissolution Testing and Organic Impurities

### 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, 1,000 mL

**Tolerances:** NLT 75% 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 1,000 mL.

**Solution A:** Mix 100 mL of acetonitrile and 100 mL of *Buffer*. Dilute with water to 1,000 mL.

**Solution B:** Mix 800 mL of acetonitrile and 10 mL of *Buffer*. Dilute with water to 1,000 mL.

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

**Mobile phase:** See table above.

**Diluent:** Prepare as directed in Identification Test A.

**System suitability stock solution:** 1 mg/mL each of USP Omeprazole and USP Omeprazole Related Compound A in methanol

**System suitability solution:** 1 µg/mL each of USP Omeprazole and USP Omeprazole Related Compound A, from the *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 seconds. Add 40 mL of *Diluent*, shake for 30 seconds 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.*

## Organic Impurities

### Chromatographic System

(See USP General Chapter 621, Chromatography, 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 impurities table 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

| Name | RRT | Acceptance criteria, NMT (%) |
|---|---|---|
| Omeprazole sulfone[1] | 0.93 | 0.5 |
| Omeprazole | 1.0 | – |
| Any other individual impurity | – | 0.2 |
| Total impurities | – | 2 |

[1]5-Methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl] sulfonyl}-1H-benzo[d]imidazole

**Acceptance criteria:** See the Impurity Table above.

## Additional Requirements

**Packaging and storage:** Preserve in tight containers. Store at room temperature.

## USP Reference Standards

- USP Omeprazole
- USP Omeprazole Related Compound A (omeprazole sulfone; 5-Methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl}-1H-benzo[d]imidazole; $C_{17}H_{19}N_3O_4S$)

# Organic Impurities Data

## Chromatographic Conditions 1

**Column:** Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Catalogue Number 1.50469)

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

**Injection:** 20 µL

**Detection:** UV 302 nm

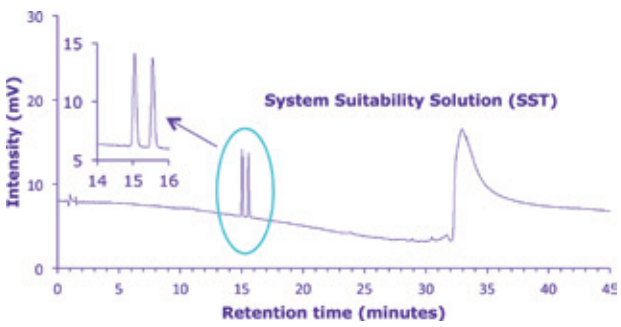
**Cell:** 10 µL

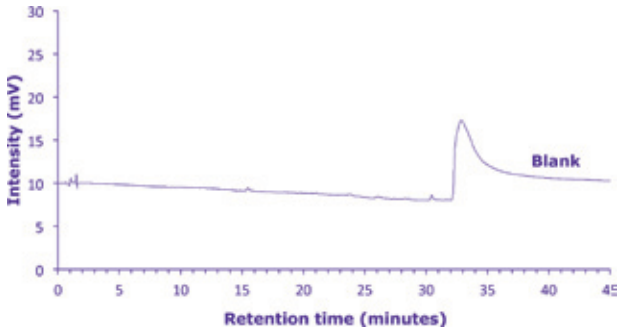
**Flow rate:** 1.0 mL/min

**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 dilute with water to 1,000 mL.

**Mobile phase**

**Solution A:** Mix 100 mL of acetonitrile and 100 mL of *Buffer*. Dilute with water to 1,000 mL.




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

| 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 |

**Solution B:** Mix 800 mL of acetonitrile and 10 mL of *Buffer*. Dilute with water to 1,000 mL.

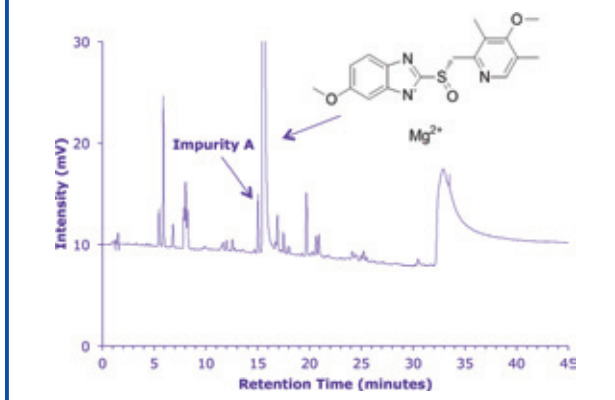
**Gradient:** See table.

**Temperature:** 25 °C

**Diluent:** Prepare a pH 11.0 diluent as follows. Dissolve 5.24 g of tribasic sodium phosphate dodecahydrate in water. Add 110 mL of 0.5 M dibasic sodium phosphate solution, and dilute with water to 1,000 mL.

**Standard solution:** Dissolve 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, and shake for 30 seconds. Add 40 mL of Diluent, shake for 30 seconds 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.




Suitability Requirements
**Resolution:** NLT 2.5 between omeprazole and its related compound A
**Relative retention time (RRT):** 0.8 for omeprazole related compound A and 1.0 for omeprazole

**Pressure drop:** 149–95 Bar (2160–1378 psi)

# Dissolution Testing Data

## Chromatographic Conditions 1

**Column:** Purospher® STAR RP-18 endcapped (5 µm) 150 x 4.6 mm (Catalogue Number 1.51455)

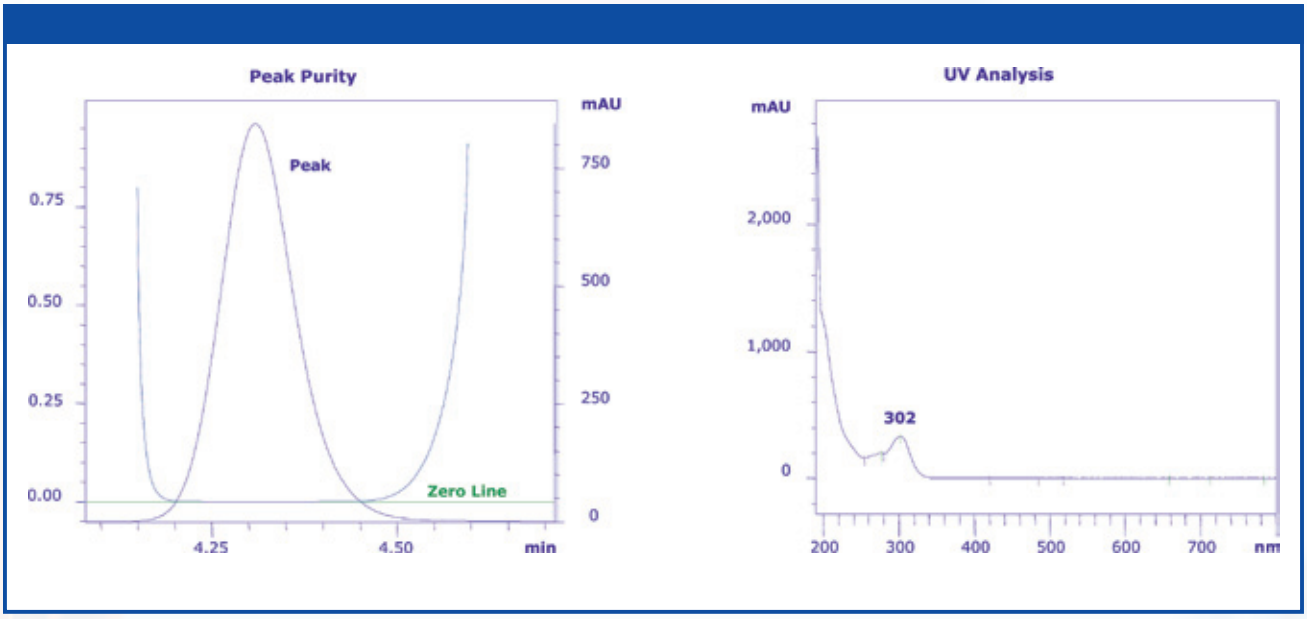
**Injection:** 20 µL

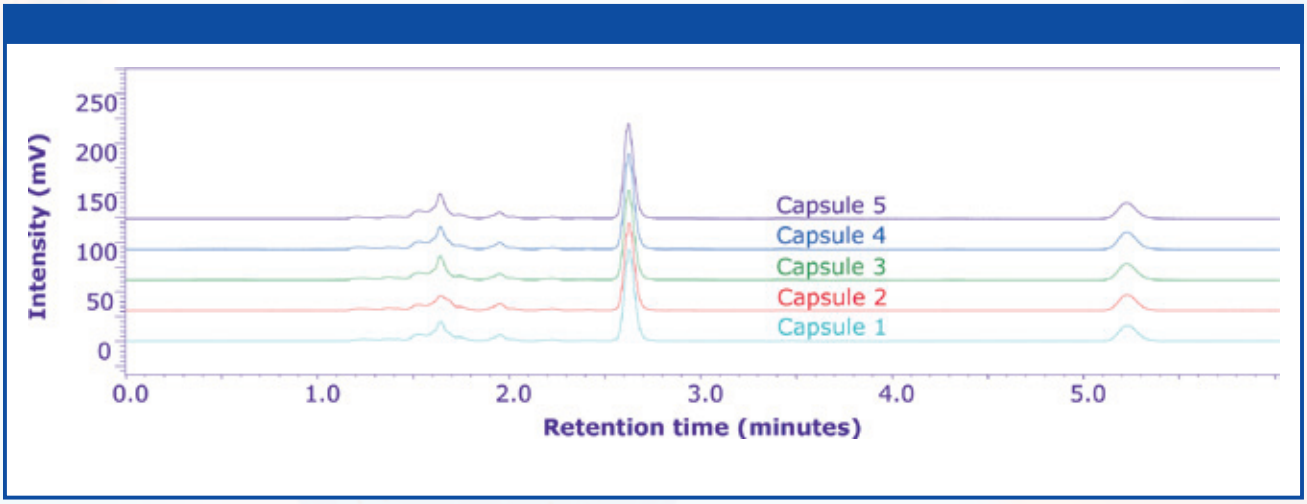
**Detection:** UV 302 nm

**Cell:** 10 µL

**Flow rate:** 1.0 mL/min

**Medium:** 0.1 N hydrochloric acid (HCl); 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 HCl 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:** Mix 350 mL of acetonitrile and 500 mL of *Buffer*. Dilute with water to 1,000 mL.

**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 dilute with water to 1,000 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 1,000 mL.

**Standard solution:** Transfer 10 mg of USP Omeprazole 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 test solution 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 (2,160 psi)

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*, 1,000 mL

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

**Dissolution Testing Data**

| Sample (area units) | Standard (area units) | Standard solution (mg/ml) | Label claim (mg/capsule) | Media volume (ml) | Dissolution (%) |
|---|---|---|---|---|---|
| 318,234 | 357,635 | 0.041 | 40 | 1,000 | 91.2 |
| 312,926 | | | | | 89.7 |
| 316,158 | | | | | 90.6 |
| 313,776 | | | | | 89.9 |
| 311,351 | | | | | 89.2 |
| Mean | | | | | 90.1 |

## Chromatographic Conditions 2

**Column:** Chromolith® HighResolution RP-18 endcapped 100 x 4.6 mm (Catalogue Number 1.52022)

**Injection:** 5 µL *(Note: 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 (HCl); 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 HCl 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:** 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 dilute with water to 1,000 mL. Mix 350 mL of acetonitrile and 500 mL of *Buffer*. Dilute with water to 1,000 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 1,000 mL.

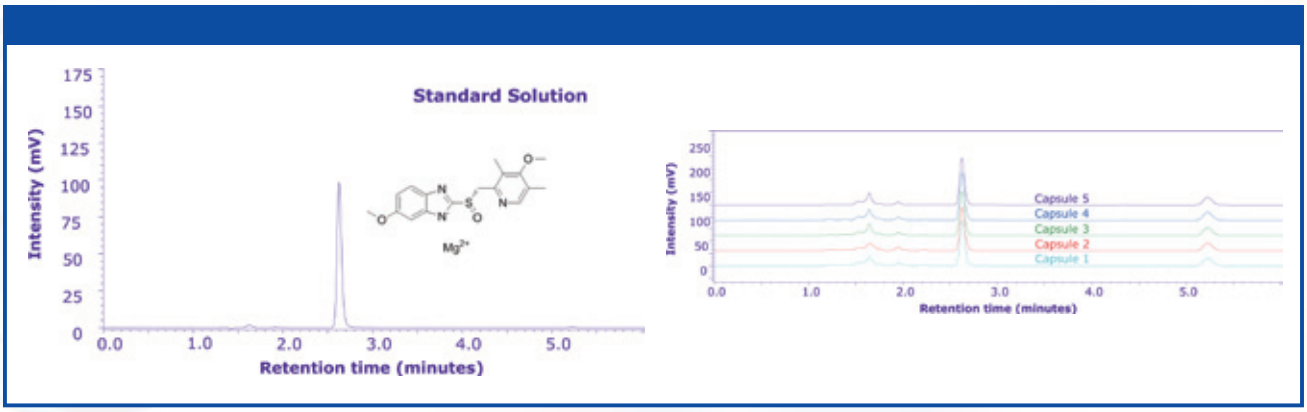
**Standard solution:** Transfer 10 mg of USP Omeprazole to a 250 mL volumetric flask, and dissolve it in about 10 mL of alcohol. Add 40 mL of Diluent, and dilute with water to volume.

**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 (1,080 psi)

**Dissolution Testing Data**

| Sample (area units) | Standard (area units) | Standard solution (mg/ml) | Label claim (mg/capsule) | Media volume (ml) | Dissolution (%) |
|---|---|---|---|---|---|
| 671,494 | 751,234 | 0.041 | 40 | 1,000 | 91.6 |
| 656,845 | | | | | 89.6 |
| 665,258 | | | | | 90.8 |
| 658,643 | | | | | 89.9 |
| 655,000 | | | | | 89.3 |
| Mean | | | | | 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*, 1,000 mL

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

## Recommended Products

### Identification—FTIR <197K>

- Potassium bromide for IR spectroscopy—Uvasol® (Catalogue Number 1.04907)

### Content of Magnesium—AAS

- Lanthanum (III) oxide for atomic absorption spectroscopy (Catalogue Number 1.10982)
- Hydrochloric acid 30%—Ultrapur (Catalogue Number 1.01514)
- Water for chromatography—LiChrosolv® (Catalogue Number 1.15333) or fresh water from the Milli-Q® system

### HPLC Assay and Related Substances (API)

- Purospher® STAR RP-8 endcapped (5 µm) 150 x 4.6 mm (Catalogue Number 1.51453) for HPLC Assay and RS analysis
- Purospher® STAR RP-8 endcapped (2 µm) 100 x 2.1 mm (Catalogue Number 1.50629) for RS analysis
- Chromolith® High Resolution RP-18 endcapped 100 x 4.6 mm (Catalogue Number 1.52022) for RS analysis
- Sodium dihydrogen phosphate dihydrate for analysis—EMSURE® Reag. Ph. Eur. (Catalogue Number 1.06342)
- Di-sodium hydrogen phosphate dihydrate for analysis—EMSURE® (Catalogue Number 1.06580)
- Tri-sodium phosphate dodecahydrate for analysis—

EMSURE® ACS, Reag. Ph. Eur. (Catalogue Number 1.06578)
- Ortho-phosphoric acid 85% for analysis—EMSURE® ACS, ISO, Reag. Ph. Eur. (Catalogue Number 1.00573)
- Acetonitrile (isocratic grade for liquid chromatography)—LiChrosolv® (Catalogue Number 1.14291)
- Water for chromatography—LiChrosolv® (Catalogue Number 1.15333) or fresh water from the Milli-Q® system

## Water Determination—Karl Fischer <921> - Ia

- CombiTitrant 5 one-component reagent for volumetric KF titration 1 ml = ca. 5 mg $H_2O$—Aquastar™ (Catalogue Number 1.88005)
- CombiSolvent methanol-free for volumetric KF titration with one component reagents—Aquastar™ (Catalogue Number 1.88008)

## HPLC Assay and Related Substances (Delayed-Release Capsules)

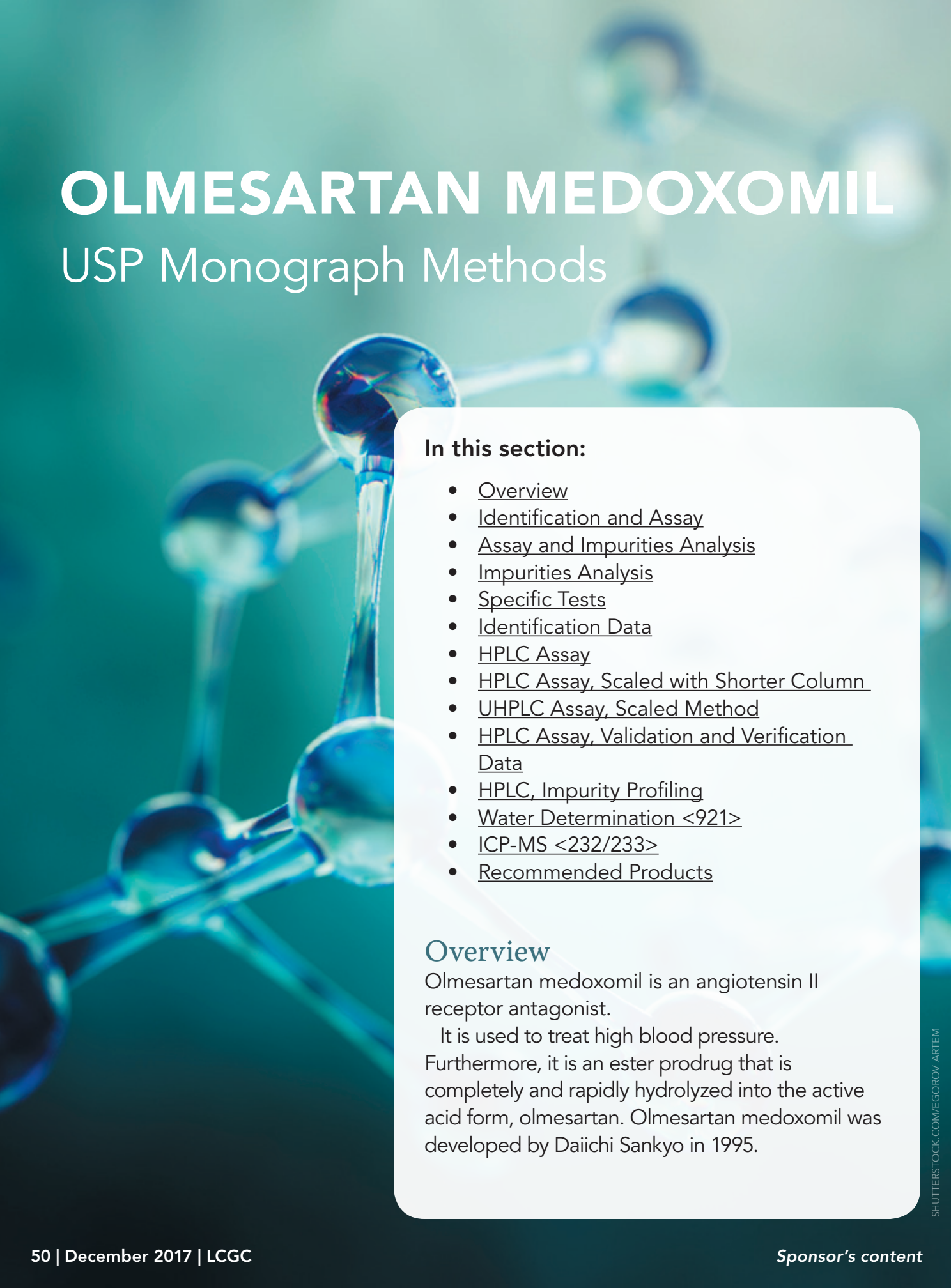
- Purospher® STAR RP-18 endcapped (5 µm) 150 x 4.6 mm (Catalogue Number 1.51455) for assay and dissolution testing
- Chromolith® HighResolution RP-18 endcapped 100 x 4.6 mm (Catalogue Number 1.52022)
- Purospher® STAR RP-18 endcapped (3 µm) 100 x 4.6 mm (Catalogue Number 1.50469) for RS analysis
- Sodium dihydrogen phosphate dihydrate for analysis—EMSURE® Reag. Ph. Eur. (Catalogue Number 1.06342)
- Di-sodium hydrogen phosphate dihydrate for analysis—EMSURE® (Catalogue Number 1.06580)
- Acetonitrile (isocratic grade for liquid chromatography)—LiChrosolv® (Catalogue Number 1.14291)
- Acetonitrile (gradient grade for liquid chromatography)—LiChrosolv® Reag. Ph. Eur. (Catalogue Number 1.00030)
- Water for chromatography—LiChrosolv® (Catalogue Number 1.15333) or fresh water from the Milli-Q® system

## Dissolution Testing (Delayed-Release Capsules)

- Hydrochloric acid (fuming 37%) for analysis—EMSURE® ACS, ISO, Reag. Ph. Eur. (Catalogue Number 1.00317)
- Sodium dihydrogen phosphate dihydrate for analysis—EMSURE® Reag. Ph. Eur. (Catalogue Number 1.06342)
- Di-sodium hydrogen phosphate dihydrate for analysis—EMSURE® (Catalogue Number 1.06580)
- Sodium hydroxide solution 50% for analysis—EMSURE® (Catalogue Number 1.58793)
- Water for chromatography—LiChrosolv® (Catalogue Number 1.15333) or fresh water from the Milli-Q® system
- Millex PTFE filter

# OLMESARTAN MEDOXOMIL

## USP Monograph Methods



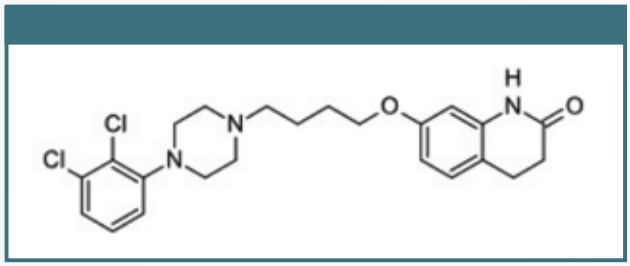
**In this section:**



## Overview

Olmesartan medoxomil is an angiotensin II receptor antagonist.

It is used to treat high blood pressure. Furthermore, it is an ester prodrug that is completely and rapidly hydrolyzed into the active acid form, olmesartan. Olmesartan medoxomil was developed by Daiichi Sankyo in 1995.




**Common commercial brand names:**
Benicar (U.S.); Olmetec (E.U., Canada, and Japan); and WinBP, Olsar, and Golme (India)

In this compilation, we have used the USP 40–NF 35 experimental conditions for olmesartan medoxomil in the following areas:

- **Identification—**FTIR
- **Assay—**HPLC and UHPLC (isocratic methods)
- **Related Substances—**HPLC (gradient method)
- **Water Determination—**Karl Fischer

The assay and related substances methods were carried out using C-18 and C-8 columns, respectively. The assay method is isocratic and has been scaled to other column dimensions with different particle sizes. We have also included a nonpharmacopeial LC-MS method for the analysis of olmesartan medoxomil related substances as well as a proposal for heavy metal analysis (using ICP-OES or ICP-MS analysis) per suggestions in the new USP General Chapters 232 and 233.

## Identification and Assay

### Definition

Olmesartan medoxomil contains not less than (NLT) 98.5% and not more than (NMT) 101.5% of C29H30N6O6, calculated on the anhydrous and solvent-free basis.

### Identification—FTIR <197K>

A. Infrared absorption
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 (isocratic isocratic method)

**Procedure:**
*Note: The Standard solution and Sample solution are stable for 24 h at 5 °C.*

**Diluted phosphoric acid:** 0.2% phosphoric acid

**Buffer:** 0.015 M monobasic potassium phosphate. Adjust the solution with diluted diluted 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 USP General Chapter 621, Chromatography, System Suitability.

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

We have used Purospher® STAR RP-18 endcapped (5 µm) 150 × 4.6 mm (Catalogue Number 1.51455).

## Assay and Impurities Analysis

### System Suitability

**Sample:** *Standard solution*

### Suitability Requirements

**Resolution:** NLT 4 between olmesartan medoxomil and p-hydroxy-benzoic 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

A. **Residue on Ignition**—USP General Chapter 281: NMT 0.1%. Note: The ignition temperature range is 450 °C to 550 °C.

B. **Heavy Metals, Method II**—USP General Chapter 231: NMT 10 ppm

| Assay and Impurities Analysis | | |
|---|---|---|
| Time (min) | Solution A (%) | Solution B (%) |
| 0 | 75 | 25 |
| 10 | 75 | 25 |
| 35 | 0 | 100 |
| 45 | 0 | 100 |

### Organic Impurities HPLC (gradient method)

- **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.

## Impurities Analysis

**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

### Chromatographic System

See USP General Chapter 621, Chromatography, 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 °C
Flow rate: 1 mL/min
Injection size: 10 µL

| | | | |
|---|---|---|---|
| Name | Relative retention time (RRT) | Relative response factor (RRF) | Acceptance criteria, NMT (%) |
| Olmesartan[1] | 0.2 | 1.0 | 0.5 |
| Olmesartan medoxomil related compound A[2] | 0.7 | 1.6 | 0.1 |
| Olmesartan medoxomil | 1.0 | 1.0 | – |
| Olefinic impurity[3] | 1.6 | 1.0 | 0.6 |
| N-alkyl impurity[4] | 3.4 | 0.7 | 0.1 |
| Any other individual unidentified impurity | – | 1.0 | 0.1 |

[1]1-{[2′-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4-(2-hydroxypropan-2-yl)-2-propyl-1H-imidazole-5-carboxylic acid
[2]1-{[2′-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1H-furo[3,4-d]imidazol-6(4H)-one
[3](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
[4](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

### System Suitability
**Sample:** *System suitability solution*

### Suitability Requirements
- **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.

**Total impurities:** NMT 1.3%.
Note: Disregard any peak below 0.05%.

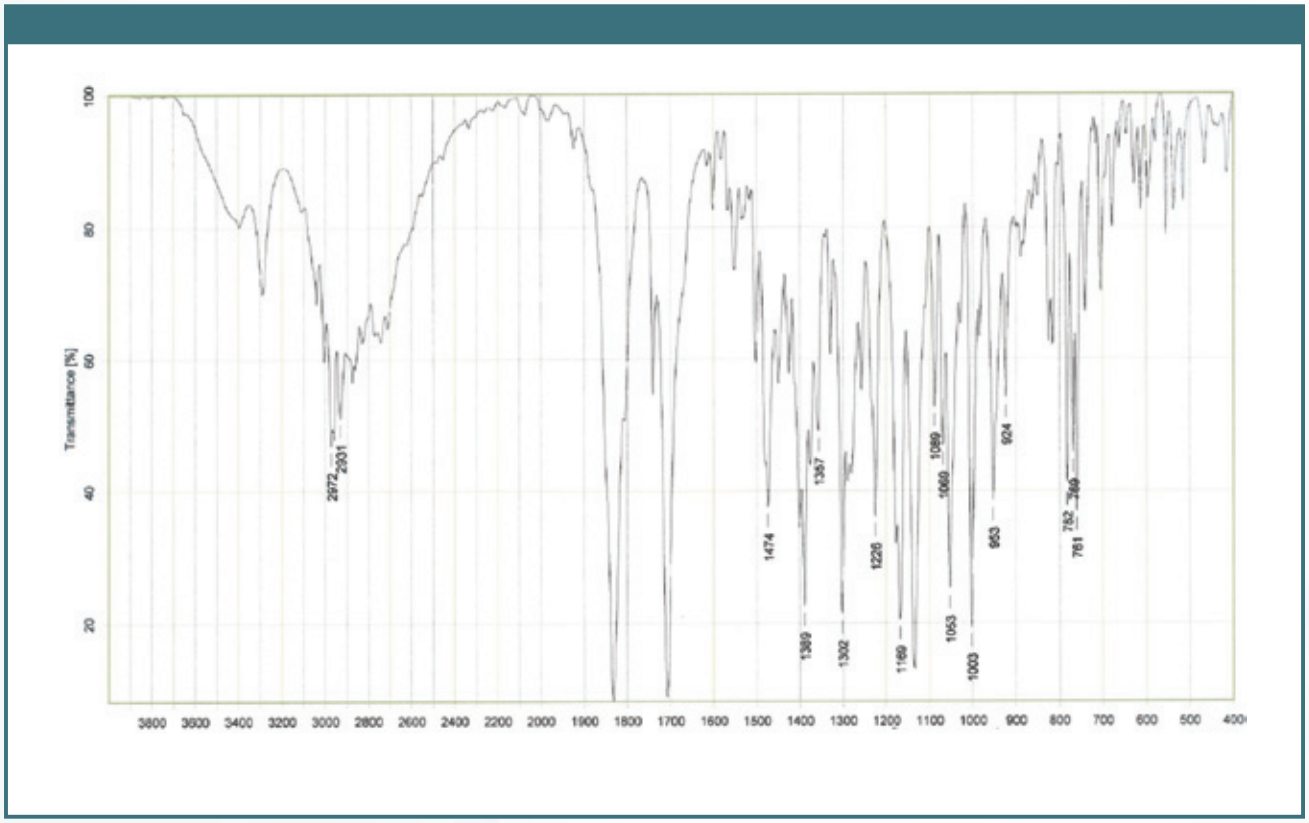
## Specific Tests
**Limit of Acetone (if present):** This test was not conducted because we only performed an analysis of the USP Reference Standards.

**Water Determination—Karl Fischer <921> - Ic:** NMT 0.5%

### Additional Requirements
**Packaging and storage:** Preserve in well-closed containers, protect from moisture, and store below 25 °C.

### 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

## Identification Data
### A. Infrared Absorption FTIR <197K>
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® (Catalogue Number 1.04907).




## HPLC Assay

**Column:** Purospher® STAR RP-18 endcapped (5 µm) 150 × 4.6 mm (Catalogue Number 1.51455)

**Injection:** 10 µL

**Detection:** UV 250 nm

**Cell:** 11 µL

**Flow rate:** 1 mL/min

**Buffer:** 0.015 M monobasic potassium phosphate, pH 3.4

**Mobile phase:** *Buffer and acetonitrile* (33:17 v/v) Acetonitrile—gradient grade (Catalogue Number 1.00030)

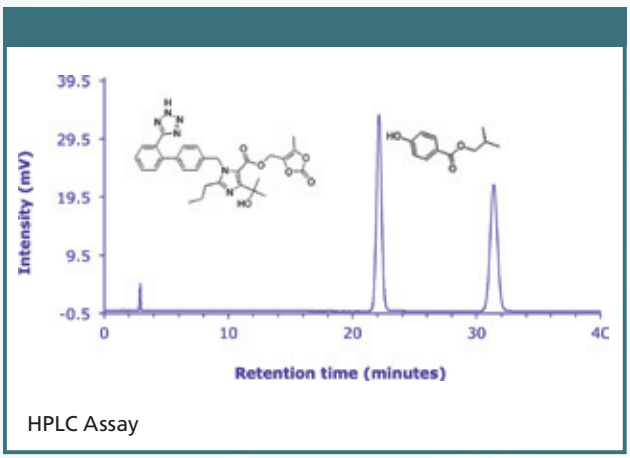
**Temperature:** 40 °C

**Diluent 1:** Acetonitrile and water (4:1)

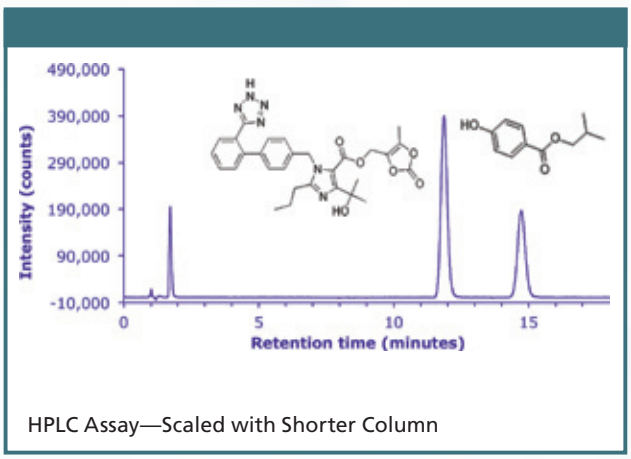
**Diluent 2:** Acetonitrile and water (2:3)

**Standard solution:** 0.05 mg/mL of USP Olmesartan Medoxomil RS of the *Standard Stock solution* and 0.025 mg/mL of p-hydroxybenzoic acid isobutyl ester from the Internal *standard solution* in *Diluent 2*

| Chromatographic Data (HPLC Assay) | | | | |
|---|---|---|---|---|
| **Compound** | **Retention time (min)** | **Resolution** | **Plates** | **Tailing factor** |
| t0 void volume | 2.9 | – | – | – |
| Olmesartan medoxomil | 22.1 | – | 13,101 | 1.0 |
| p-HBA i-Butyl ester | 31.3 | 9.8 | 12,822 | 1.0 |



HPLC Assay



HPLC Assay—Scaled with Shorter Column

**Internal standard solution:** 0.5 mg/mL of p-hydroxybenzoic acid isobutyl ester in *Diluent 2*

**Stock solution:** 1 mg/mL of USP Olmesartan Medoxomil RS in *Diluent 1*

**Pressure drop:** 63 Bar (907 psi)

**Suitability Requirements**
**Resolution:** NLT 4 between olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester

## HPLC Assay—Scaled with Shorter Column

**Column:** Purospher® STAR RP-18 endcapped (3 µm) 100 × 2.1 mm (Catalogue Number 1.50653)

**Injection:** 2.1 µL

**Detection:** UV 250 nm

**Cell:** 11 µL

**Flow rate:** 1.0 mL/min

**Buffer:** 0.015 M monobasic potassium phosphate, pH 3.4

**Mobile phase***: Buffer* and acetonitrile (33:17 v/v)
Acetonitrile—gradient grade (Catalogue Number 1.00030)

**Temperature:** 40 °C

**Diluent 1:** Acetonitrile and water (4:1)

| Chromatographic Data (Scaled with Shorter Column) | | | | |
|---|---|---|---|---|
| Compound | Retention time (min) | Resolution | Plates | Tailing factor |
| t0 void volume | 1.3 | – | – | – |
| Olmesartan medoxomil | 11.9 | – | 11,270 | 1.1 |
| p-HBA i-Butyl ester | 14.7 | 5.7 | 11,345 | 1.1 |

**Diluent 2:** Acetonitrile and water (2:3)

**Standard solution:** 0.05 mg/mL of USP Olmesartan Medoxomil RS of the *Standard Stock solution* and 0.025 mg/mL of p-hydroxybenzoic acid isobutyl ester from the Internal *standard solution* in *Diluent 2*

**Internal standard solution:** 0.5 mg/mL of p-hydroxybenzoic acid isobutyl ester in *Diluent 2*

**Stock solution:** 1 mg/mL of USP Olmesartan Medoxomil RS in *Diluent 1*

**Pressure drop:** 76 Bar (1102 psi)

**Suitability Requirements**
**Resolution:** NLT 4 between olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester

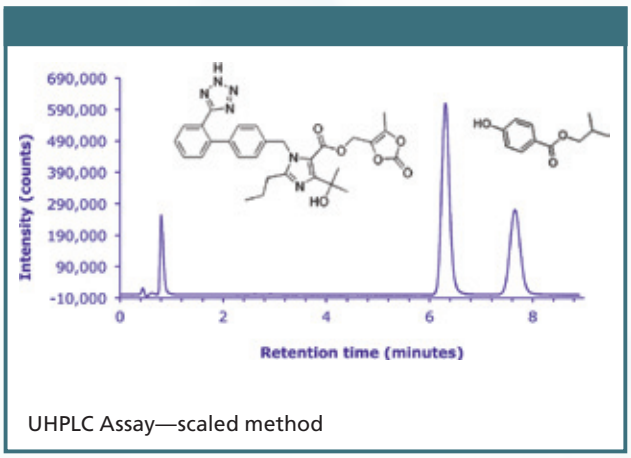
## UHPLC Assay—Scaled Method

**Column:** Purospher® STAR RP-18 endcapped (2 µm) 50 × 2.1 mm (Catalogue Number 1.50646)

**Injection:** 2.1 µL

**Detection:** UV 250 nm

**Cell:** 1.4 µL




UHPLC Assay—scaled method

**Flow rate:** 0.2 mL/min

**Buffer:** 15 mM monobasic potassium phosphate, pH 3.4

**Solution B:** Acetonitrile—gradient grade (Catalogue Number 1.00030)

**Mobile phase:** *Buffer* and acetonitrile (33:17 v/v)

**Temperature:** 40 °C

**Diluent 1:** Acetonitrile and water (4:1)

**Diluent 2:** Acetonitrile and water (2:3)

**Standard solution:** 0.05 mg/mL of USP Olmesartan Medoxomil RS of the *Standard stock solution* and 0.025 mg/mL of

| Chromatographic Data (UHPLC Assay) | | | | |
|---|---|---|---|---|
| Compound | Retention time (min) | Resolution | Plates | Tailing factor |
| t0 void volume | 0.7 | – | – | – |
| Olmesartan medoxomil | 6.4 | – | 7,962 | 1.1 |
| p-HBA i-Butyl ester | 7.8 | 4.0 | 6,527 | 1.1 |

p-hydroxybenzoic acid isobutyl ester from the Internal *standard solution* in *Diluent 2*

**Stock solution:** 1 mg/mL of USP 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

## HPLC Assay—Validation and Verification Data

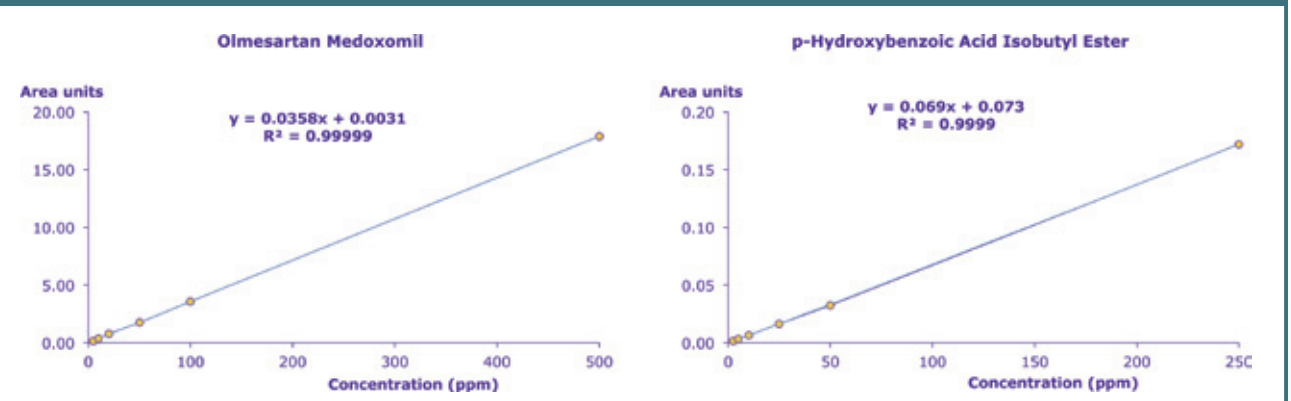
### 1. Specificity

Determined by injection of *System suitability solution* and determination of the retention time and relative retention time for Olmesartan Medoxomil A RS and USP Olmesartan Medoxomil RS using a Purospher® STAR RP-18 endcapped (5 µm) 150 × 4.6 mm column

**System Suitability Criteria**
**Resolution:** NLT 4 between olmesartan medoxomil and p-hydroxybenzoic acid isobutyl ester

| | | | | |
|---|---|---|---|---|
| Compound | Retention time (min) | RRT | Tailing factor | Resolution |
| Olmesartan medoxomil | 22.1 | 1.0 | 1.0 | – |
| p-HBA i-Butyl ester | 31.4 | 1.4 | 1.0 | 9.8 |

| | | | | |
|---|---|---|---|---|
| | 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 | |




### 2. Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Determined by injecting six concentration levels from5–500 ppm of USP Olmesartan Medoxomil RS and six concentration levels ranging from 2.5–250 ppm of p-hydroxybenzoic acid isobutyl ester

## HPLC—Impurity Profiling

### Purospher® STAR RP-8 endcapped (3 µm)

**Column:** Purospher® STAR RP-8 endcapped (3 µm) 100 × 4.6 mm (Catalogue Number 1.50013 customized packing)

**Injection:** 10 µL

**Detection:** UV 250 nm

**Cell:** 11 µL
**Flow rate:** 1 mL/min

**Solution A:** 15 mM monobasic potassium phosphate, pH 3.4

**Solution B:** Acetonitrile—gradient grade (Catalogue Number 1.00030)

**Mobile phase:**
A : *Solution A and Solution B* (4:1 v:v)
B : *Solution A and Solution B* (1:4 v:v)

**Gradient:** See table.

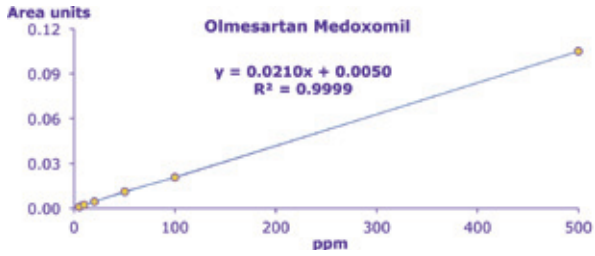
**Temperature:** 40 °C

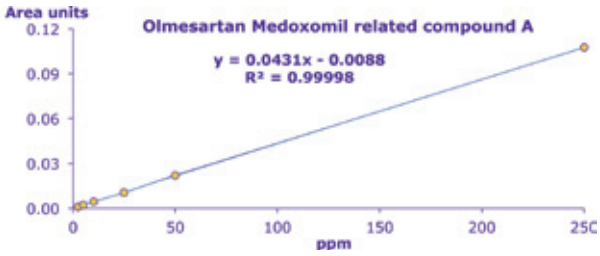
**Diluent:** Acetonitrile

**Impurity standard:** 0.01 mg/mL of USP Olmesartan Medoxomil RS in acetonitrile

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

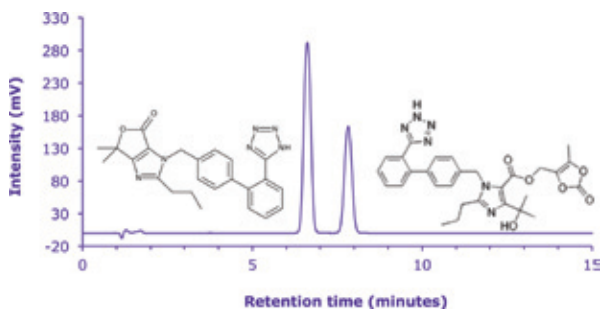
**Impurity sample:** 1 mg/mL of USP Olmesartan Medoxomil RS in acetonitrile




Validation and verification data

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




HPLC—impurity profiling

**Pressure drop:** 48–108 Bar (696–1566 psi)

**Suitability Requirements**

**Resolution:** NLT 5 between olmesartan medoxomil and olmesartan medoxomil related compound A

## HPLC Impurity Profiling—Validation and Verification

### 1. Specificity

Determined by injection of *System suitability solution* and determination of the retention time and relative retention time for olmesartan medoxomil related compound A and olmesartan medoxomil using a Purospher® STAR RP-8 endcapped (3 µm) 100 × 4.6 mm column

| Compound | Retention time (min) | Resolution | Plates | Tailing factor |
|---|---|---|---|---|
| t0 void volume | 1.3 | – | – | – |
| Olmesartan medoxomil related compound A | 6.6 | – | 5,004 | 1.0 |
| Olmesartan medoxomil | 7.8 | 5.7 | 5,926 | 1.0 |

| Compound | Retention time (min) | Resolution | Plates | Tailing factor |
|---|---|---|---|---|
| Olmesartan medoxomil related compound A | 6.6 | 0.85 | 1.0 | – |
| Olmesartan medoxomil | 7.8 | 1.0 | 1.0 | 5.7 |

| | Olmesartan medoxomil (ppm) | Area (mAU*min) | Olmesartan medoxomil related compound 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 | |

### 2. Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Determined by injecting six concentration levels from 5–500 ppm of USP Olmesartan Medoxomil RS and six concentration levels ranging from 2.5–250 ppm of USP Olmesartan Medoxomil Related Compound A RS

## HPLC—Impurity Profiling

### LC-MS-Compatible Method for Olmesartan and Related Substances (Nonpharmacopeial Method)

In the following sections, 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-18 endcapped (2 µm) 100 × 2.1 mm (Catalogue number 1.506546)

**Injection:** 0.3 µL

**Detection:** Pos. ESI-MS (MRMs 559->541; 429->207)

**Flow Rate:** 210 µL/min

**Mobile phase:** Acetonitrile and buffer 1:3 (v/v)

**Buffer:** 0.015 M ammonium acetate, pH adjusted to 3.4 with glacial acetic acid

**Temperature:** 40° C

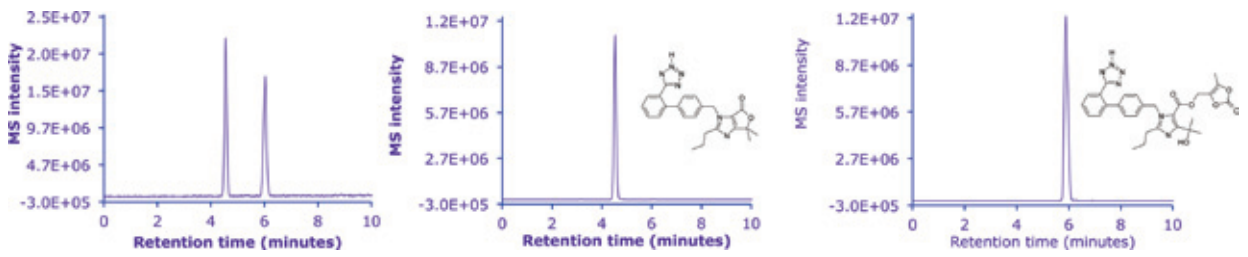
**Diluent:** Acetonitrile

**Sample:** 0.01 mg/mL each of Olmesartan medoxomil RS and related compound A in Acetonitrile (system suitability solution)

**Pressure Drop:** 51-102 Bar (734–1469 psi)

**Suitability Criteria:** Chromatographic

| Compound | Retention Time (min) | Resolution | Tailing factor | Molecular Weight | m/z | MRM transition |
|---|---|---|---|---|---|---|
| Olmesartan RS A | 4.6 | – | 1.1 | 428.2 | 429.2 | 429 → 206 |
| Olmesartan | 6.0 | >>5 | 1.1 | 558.2 | 559.4 | 559 → 541 |




Validation and verification data

| | Olmesartan medoxomil (ppm) | Area Counts | Olmesartan medoxomil A (ppm) | Area Counts |
|---|---|---|---|---|
| | 1.9 | 1,427,570 | 2.2 | 13,616,131 |
| | 5.8 | 52,720,141 | 6.6 | 39,221,377 |
| | 7.7 | 71,536,517 | 8.8 | 54,139,425 |
| | 9.7 | 88,994,869 | 11.0 | 69,691,922 |
| | 11.6 | 110,832,984 | 13.2 | 82,201,653 |
| | 15.5 | 146,305,786 | 17.7 | 111,025,202 |
| | 19.3 | 179,311,621 | 22.1 | 137,770,528 |
| STEYX | | 1,876,144 | | 1,004,073 |
| SLOPE | | 9,530,333 | | 6,308,188 |
| LOD | 0.6 | | 0.5 | |
| LOQ | 1.9 | | 1.6 | |

resolution not less than (NLT) 5 between Olmesartan and Olmesartan RS A

### 1. Specificity

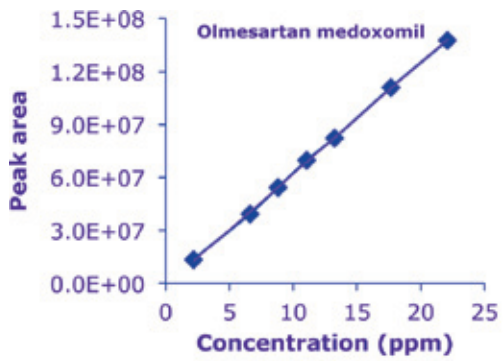
Determined by injection of the *system suitability solution* and monitoring the retention time and relative retention time for Olmesartan medoxomil RS A and Olmesartan medoxomil using a Purospher® STAR RP-88 endcapped (2 µm) 100 × 2.1 mm column.

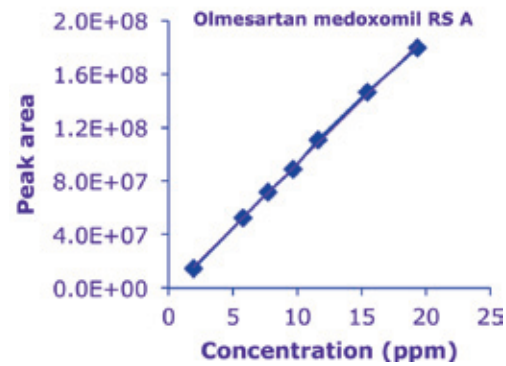
### 2. Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

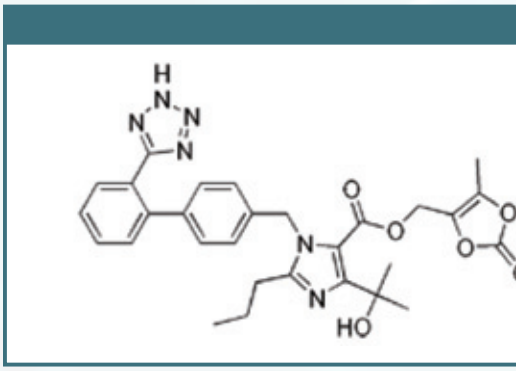
Determined by injecting seven concentration levels from 1.5–25.0 ppm of Olmesartan medoxomil and Olmesartan medoxomil RS A.

## Water Determination <921>

Pharmaceutical products sometimes involve complex formulations. In pharmaceutical guidelines (USP, Ph.




Eur., DAB), Karl Fischer (KF) titration is described as a common method for water determination. However, for certain substances, there are specialized procedures. Difficulties associated with Karl Fischer determination are often caused by limited solubility. In some cases, depending upon the composition and properties of the formulations, it is necessary to consider side reactions. The determination of mass loss as method for water determination is not recommended.

In the case of olmesartan medoxomil, the water determination can be carried out without difficulty using standard methods.

## Titration—One-Component System

**Working medium:** CombiCoulomat fritless KF reagent for coulometric water determination (for cells with and without diaphragm)—Aquastar™ (Catalogue Number 1.09257)

## Titration Parameters

**Stirring time:** 60 s

**Default coulometer settings for cell without diaphragm (e.g., for endpoint indication,):** 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 USP Olmesartan Medoxomil RS.)

## Procedure

Place the Karl Fischer reagent into the titration cell without diaphragm. Start the coulometer and titrate the solvent dry. After preliminary titration and stabilization of drift, add the sample into the titration cell with a weighing boat (for exact

| Oral dose | | | Parenteral dose | | |
|---|---|---|---|---|---|
| Element | | PDE[1] (µg/day) | Element | | PDE[1] (µg/day) |
| 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 | 1,000 | Copper | Cu | 100 |
| Molybdenum | Mo | 100 | Molybdenum | Mo | 10 |
| Nickel | Ni | 500 | Nickel | Ni | 50 |
| Vanadium | V | 100 | Vanadium | V | 10 |

[1]PDE: Permissible daily dose based on a person of 50 kg

sample weight determination, weigh the weighing boat before and after injection) and begin the water determination. (For complete dissolution of the sample, we recommend a stirring time of 60 seconds.)

### Result

**Measured water content in olmesartan:** 0.054% (USP requirement: <0.5%)

## ICP-MS <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

Digest a 0.1 g sample (closed microwave digestion) in 3 mL $HNO_3$ with 1 mL HCl and 2 mL $H_2O_2$.

### Calibration

(using ICP multielement standards)The impurities were tested for both oral and parenteral dosage. Thus, the calibration of the high-resolution inductively-coupled mass spectrometry (HR-ICP-MS) was performed for oral and parenteral dosage.

### The Limits of Impurities

- For oral dose, the ICP multielement standards were used.
- The multielement standard 5.05101 that contains Cd, Pb, As, Hg, Cu, Mo, Ni, V was diluted in nitric acid.
- The multielement standard 5.05103 that contains Ir, Os, Pd, Pt, Rh, Ru was diluted in hydrochloric acid.
- For parenteral dose, the ICP multielement standards 5.05102 and 5.05104 were used.

- The multielement standard 5.05102 that contains Cd, Pb, As, Hg, Cu, Mo, Ni, V was diluted in nitric acid.
- The multielement standard 5.05104 that contains Ir, Os, Pd, Pt, Rh, Ru was diluted in hydrochloric acid.

## Recommended Products

### Identification—FTIR <197K>

- Potassium bromide for IR spectroscopy — Uvasol® (Catalogue Number 1.04907)

### Water Determination—Karl Fischer <921> - Method Ic

- CombiCoulomat fritless KF reagent for coulometric water determination (for cells with and without diaphragm)—Aquastar™ (Catalogue Numbers 1.09257 and 1.88002)
- CombiCoulomat fritless KF reagent for coulometric water determination (for cells with and without diaphragm) — Aquastar™ (Catalogue Number 1.09257)

### Assay (HPLC) and Related Substances (HPLC)

- Purospher® STAR RP-18 endcapped (5 µm) 150 × 4.6 mm for assay (Catalogue Number 1.51455)
- Purospher® STAR RP-18 endcapped (3 µm) 100 × 2.1 mm for assay (Catalogue Number 1.50653)
- Purospher® STAR RP-18 endcapped (2 µm) 50 × 2.1 mm for assay (Catalogue Number 1.50651)
- Purospher® STAR RP-8 endcapped (3 µm) 100 × 4.6 mm for RS analysis (Catalogue Number 1.50013 customized packing)
- Sodium dihydrogen phosphate dihydrate for analysis—EMSURE® Reag. Ph. Eur. (Catalogue Number 1.06342)
- Orthophosphoric acid 85% for analysis—EMSURE® ACS, ISO, Reag. Ph. Eur. (Catalogue Number 1.00573)
- Acetonitrile (isocratic grade for LC)—LiChrosolv® (Catalogue Number 1.14291)
- Acetonitrile (gradient grade for LC)—LiChrosolv® Reag. Ph. Eur. (Catalogue Number 1.00030)
- Water for chromatography (LC-MS grade)—LiChrosolv® (Catalogue Number 1.15333) or fresh water from the Milli-Q® system

### Related Substances (LC-MS)

- Purospher® STAR RP-18 endcapped (3 µm) 100 × 2.1 mm (Catalogue Number 1.50653)
- Acetonitrile (hypergrade) for LC-MS—LiChrosolv® (Catalogue Number 1.00029)
- Formic acid 98-100% for analysis—EMSURE® ACS, Reag. Ph. Eur. (Catalogue Number 1.00264)
- Water for chromatography (LC-MS grade)—LiChrosolv® (Catalogue Number 1.15333) or water from the Milli-Q® system

### Analysis (ICP)

- Nitric acid 65%—Suprapur® (Catalogue Number 1.00441)
- Hydrochloric acid 30%—Suprapur® (Catalogue Number 1.00318)
- Hydrogen peroxide 30%—Suprapur® (Catalogue Number 1.07298)

### Elements As, Cd, Cu, Hg, Mo, Ni, Pb, V

- ICP multielement standard USP-I according to <232> oral dose—Certipur® (Catalogue Number 5.05101)
- ICP multielement standard USP-II according to <232> parenteral dose—Certipur® (Catalogue Number 5.05102)

### Elements Ir, Os, Pd, Pt, Rh, Ru

- ICP multielement standard USP-III according to <232> oral dose 100 mg/L—Certipur® (Catalogue Number 5.05103)
- ICP multielement standard USP-IV according to <232> parenteral dose 10 mg/L—Certipur® (Catalogue Number 5.05104)

# RALOXIFENE HYDROCHLORIDE

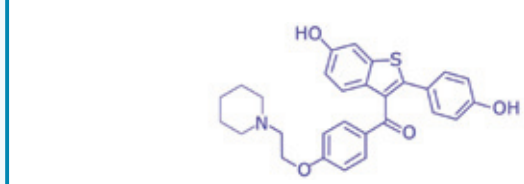
## USP Monograph Methods

**In this section:**



## Overview

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 hydrochloride was developed by Eli Lilly and Company. In 2010, sales in were $1.3 billion globally; the patent expired in 2014.

In this compilation, we have used the USP 37–NF 32 experimental conditions for raloxifene hydrochloride in the following areas:

- **Identification—**FTIR
- **Assay—**HPLC and UHPLC (isocratic methods)
- **Related Substances—**HPLC (gradient method)

The assay and related substances have been carried out with HPLC using RP-8 and RP-18 endcapped columns. In addition, the assay method has been scaled to a shorter UHPLC column dimension with a different particle size.

## Identification and Assay

### Definition

Raloxifene hydrochloride contains not less than (NLT) 97.5% and not more than (NMT) 102.0% of raloxifene hydrochloride ($C_{28}H_{27}NO_4S{\cdot}HCl$), calculated on the dried basis.

### Identification—FTIR <197K>

- Infrared absorption
- Identification Tests—USP General Chapter 191, Chloride: The sample being dissolved in methanol meets the requirements.

### Assay—HPLC and UHPLC (Isocratic Methods)

**Buffer:** Dissolve 7.2 g of monobasic potassium phosphate in 1,000 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 in *Mobile phase*

**Sample solution:** 0.05 mg/mL of raloxifene hydrochloride in Mobile phase

**Chromatographic System**

See USP General Chapter 621, Chromatography, 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 *(Note: We used 1.0 mL/min for the HPLC method and 0.21 mL/min for the UHPLC method.)*
*Injection volume:* 10 µL

**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

## Assay Analysis and Impurities Solutions

### 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 sample taken.

**Result =** (rU/rS) × (CS/CU) × 100

rU = peak response from the *Sample solution*

rS = peak response from the *Standard solution*

CS = concentration of USP Raloxifene Hydrochloride 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 1,000 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.

**Table 1**

| Time (min) | Solution A (%) | Solution B (%) |
|---|---|---|
| 0 | 75 | 25 |
| 9 | 75 | 25 |
| 40.25 | 50 | 50 |
| 42.25 | 75 | 25 |
| 49 | 75 | 25 |

**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.*

## Impurities Analysis

**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 Related Compound C in *Diluent B*

**System suitability solution:** Transfer 15 mg of USP Raloxifene Hydrochloride 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 in *Diluent A*

**Sample solution:** 3 mg/mL of raloxifene hydrochloride in *Diluent A*

### Chromatographic System

See USP General Chapter 621, Chromatography, 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.

**Result** = (rU/rS) × (CS/CU) × 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 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%.

## Related Substances (Organic Impurities)

**Table 2**

| Name | Relative retention time (RRT) | Acceptance criteria, NMT (%) |
|---|---|---|
| Raloxifene 3,7-diketone[1] | 0.74 | 0.20 |
| Raloxifene | 1.00 | – |
| Other impurities | – | 0.10 |
| Total impurities | – | 0.5 |

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

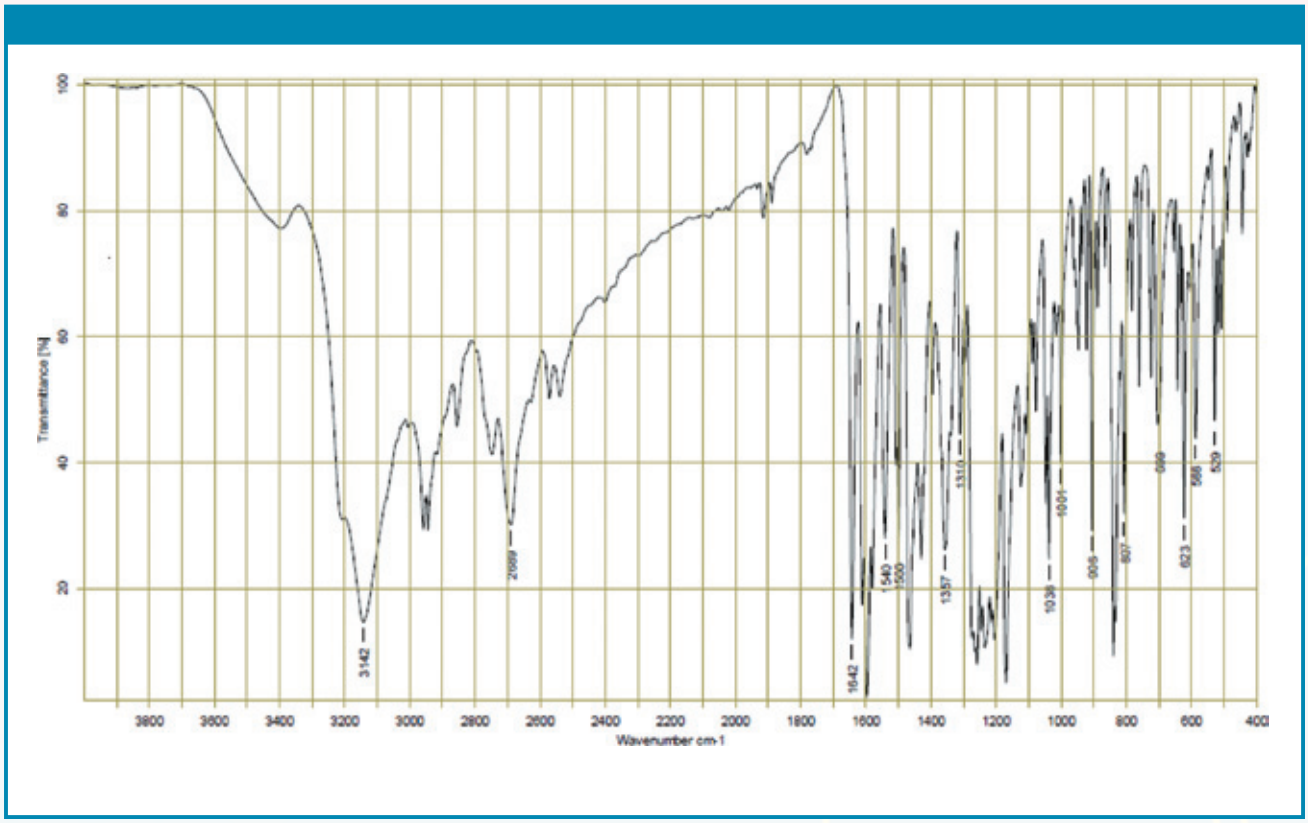
*Note: In USP 38-NF 33 it is mentioned to delete the following: Heavy Metals, Method II—USP General Chapter 231: NMT 10 ppm (official Dec. 1, 2015).*

### Additional Requirements

*Packaging and storage:* Preserve in tight containers and store at controlled room temperature.

### USP Reference Standards

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




## Indentification Data

### *Infrared Absorption*

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® (Catalogue Number 1.04907).

## Assay and Related Substances Data

### Chromatographic Conditions 1

**Column:** Purospher® STAR RP-8 endcapped (3 µm) 150 x 4.6 mm (Catalogue Number 1.50009)

**Injection:** 10 µL

**Detection:** UV 280 nm

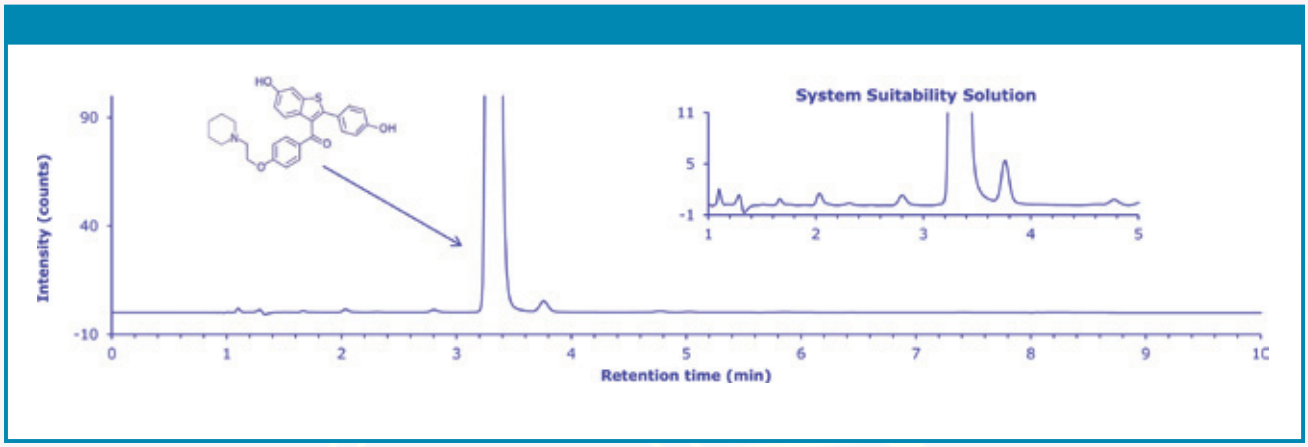
**Cell:** 11 µL

**Flow rate:** 1.5 mL/min

**Mobile phase:** Dissolve 7.2 g of monobasic potassium phosphate in 1,000 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.




**Standard solution:** 0.05 mg/mL of USP Raloxifene Hydrochloride 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 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 (2,494 psi)

**Suitability Requirements**

- **Resolution:** NLT 2.0 between raloxifene and raloxifene related compound C
- **Tailing factor:** NMT 2.0 for raloxifene

| Compound | Retention time (min) | Plates | Resolution | Tailing factor |
|---|---|---|---|---|
| t0 void volume | 1.3 | – | – | – |
| Raloxifene | 3.3 | 9,337 | – | 1.14 |
| Raloxifene related compound C | 3.8 | 11,166 | 3.4 | 1.25 |

# Assay and Related Substances Validation Data

## Chromatographic Conditions 1

### 1. Specificity

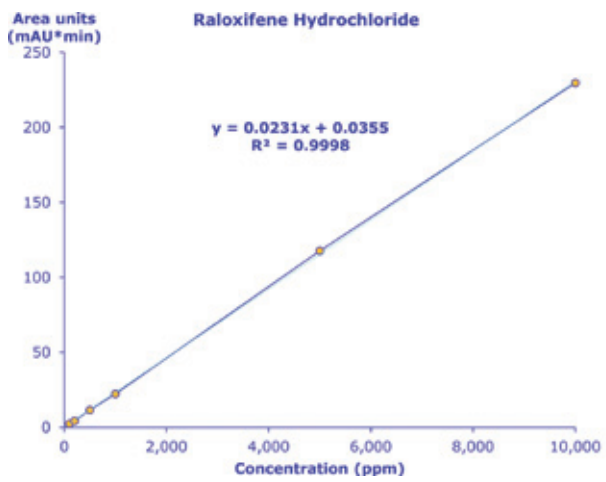
Determined by injection of *System suitability solution* and determination of the retention time and relative retention time for raloxifene hydrochloride and raloxifene related compound C using a Purospher® STAR RP-8 endcapped (3 µm) 150 x 4.6 mm column

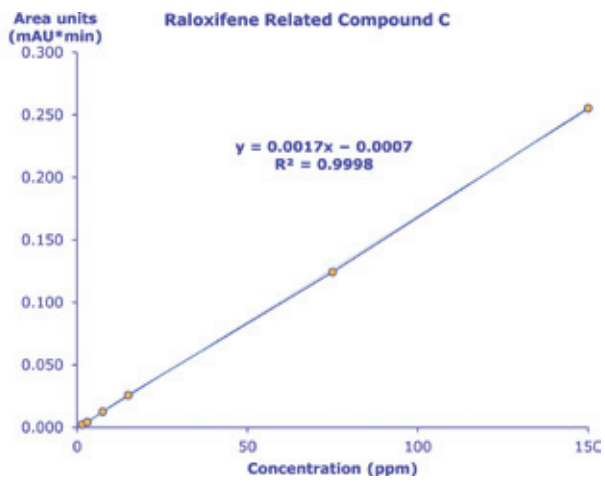
| Compound | Retention time (min) | RRT | Tailing factor | Resolution |
|---|---|---|---|---|
| Raloxifene hydrochloride | 3.34 | – | 1.16 | – |
| Raloxifene related compound C | 3.95 | 0.85 | 1.05 | 3.4 |

### 2. Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Determined by injecting six concentration levels from 100 to 10,000 ppm of raloxifene hydrochloride and six concentration levels ranging from 1.5 to 150 ppm of raloxifene related compound C

| | Raloxifene hydrochloride (ppm) | Area units (mAU*min) | Raloxifene related compound C (ppm) | Area units (mAU*min) |
|---|---|---|---|---|
| | 100 | 2.378 | 1.5 | 0.0002 |
| | 200 | 4.305 | 3.0 | 0.0004 |
| | 500 | 11.498 | 7.5 | 0.012 |
| | 1,000 | 22.221 | 15 | 0.026 |
| | 5,000 | 117.685 | 75 | 0.124 |
| | 10,000 | 229.634 | 150 | 0.255 |
| LOD | | 85 | | 1.3 |
| LOQ | | 259 | | 4.0 |




# Assay and Related Substances Data

## Chromatographic Conditions 2

**Column:** Purospher® STAR RP-8 endcapped (2 µm) 100 x 2.1 mm (Catalogue Number 1.50629.0001)

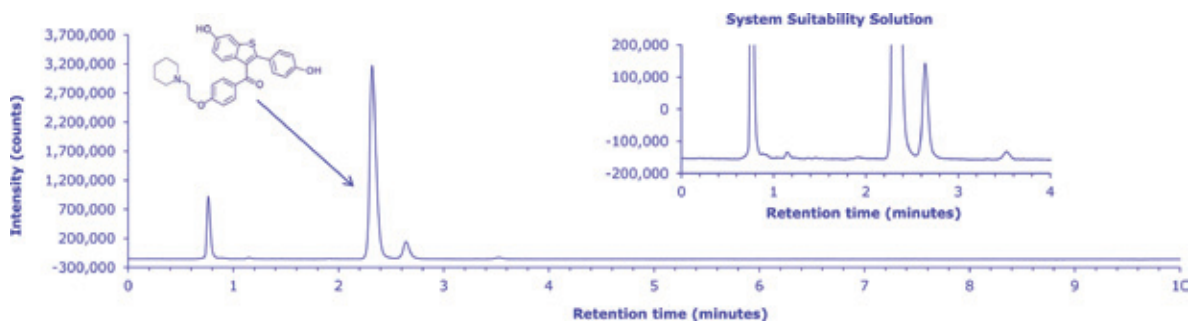
**Injection:** 2 µL

**Detection:** UV 280 nm

**Cell:** 1.4 µL

**Flow rate:** 0.21 mL/min

**Medium:** Dissolve 7.2 g of monobasic potassium phosphate in 1,000 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).




Assay and Related Substances Data

| Compound | Retention time (min) | Resolution | Plates | Tailing factor |
|---|---|---|---|---|
| t0 void volume | 0.9 | – | – | – |
| Raloxifene | 2.3 | – | 7,051 | 1.13 |
| Raloxifene related compound C | 2.6 | 2.8 | 8,119 | 1.11 |

**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.

**Standard solution:** 0.05 mg/mL of USP Raloxifene Hydrochloride 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 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 (3,785 psi)

**Suitability Requirements**

- **Resolution:** NLT 2.0 between raloxifene and raloxifene related compound C
- **Tailing factor:** NMT 2.0 for raloxifene hydrochloride

## Assay and Related Substances Validation Data

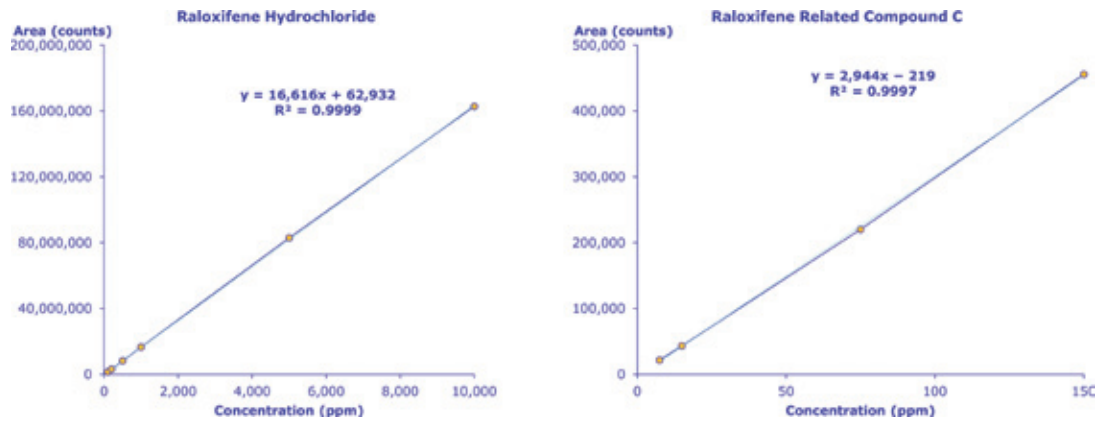
### Chromatographic Conditions 2

### 1. Specificity

| Compound | Retention time (min) | RRT | Tailing factor | Resolution |
|---|---|---|---|---|
| Raloxifene | 2.32 | – | 1.3 | – |
| Raloxifene related compound C | 2.64 | 0.88 | 1.1 | 1.2 |

Determined by injection of *System suitability solution* and determination of the retention time and relative retention

| | Raloxifene hydrochloride (ppm) | Area (counts) | Raloxifene related compound C (ppm) | Area (counts) |
|---|---|---|---|---|
| | 100 | 1,548,778 | 7.5 | 21,459 |
| | 200 | 3,093,681 | 15 | 43,233 |
| | 500 | 8,091,485 | 75 | 220,077 |
| | 1,000 | 16,499,725 | 150 | 455,731 |
| | 5,000 | 82,905,592 | | |
| | 10,000 | 162,787,912 | | |
| LOD | | 12.5 | | 0.25 |
| LOQ | | 38 | | 0.75 |




Assay and Related Substances Validation Data

time for raloxifene hydrochloride and raloxifene related compound C using a Purospher® STAR RP-8 endcapped (2 µm) 100 x 2.1 mm column

### 3. Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Determined by injecting six concentration levels from 100 to 10,000 ppm of raloxifene hydrochloride and four concentration levels ranging from 7.5 to 150 ppm of raloxifene related compound C

## Assay and Related Substances Data

### Chromatographic Conditions 3

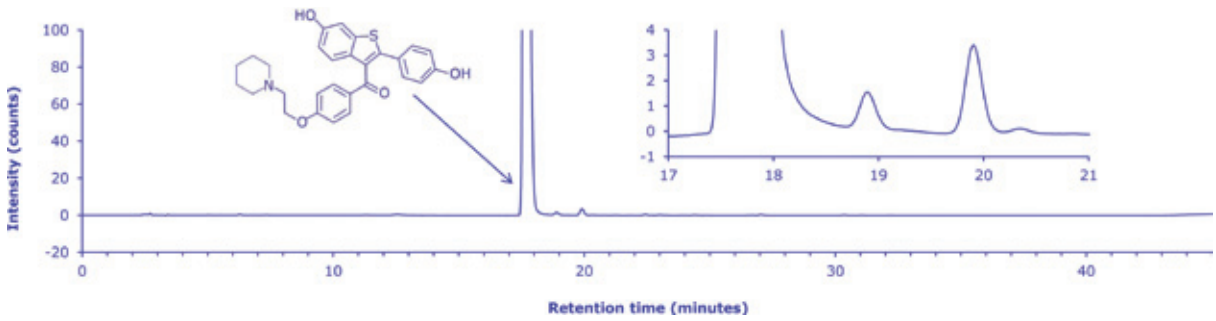
**Column:** Purospher® STAR RP-8 endcapped (5 µm) 250 x 4.6 mm (Catalogue Number 1.51454)

**Injection:** 10 µL

**Detection:** UV 280 nm

**Cell:** 11 µL

**Flow rate:** 1.5 mL/min




| Compound | Retention time (min) | RRT | Plates | Resolution | Tailing factor |
|---|---|---|---|---|---|
| t0 void volume | 3.4 | – | – | – | – |
| Raloxifene hydrochloride | 17.3 | 0.86 | 66,831 | – | 1.10 |
| Raloxifene related compound C | 19.9 | 1.0 | 72,260 | 6.1 | 1.06 |

**Solution:** *Assay solution A* and acetonitrile (75:25)

**Solution B:** *Assay solution A* and acetonitrile (50:50)

**Gradient:** See table.

**Temperature:** 35 °C

**Diluent 1:** Acetonitrile and *Buffer* (60:40)

**System suitability solution:**
Transfer 15 mg of USP Raloxifene Hydrochloride to a 50-mL volumetric flask stock and add 15 mL acetonitrile, 3 mL water, and 5 mL of 30% hydrogen peroxide. Shake the solution for 30 min and follow with 30 min sonication. Let it stand for at least 6 h at 30 °C. Dilute with *Diluent 1*[1] to 50 mL.

**System suitability solution:**
Transfer 15 mg of USP Raloxifene Hydrochloride to a 50 mL volumetric flask, then add 5 mL of System suitability stock solution and 20 mL of Diluent 2[2]. Dilute with impurity *Solution A*.

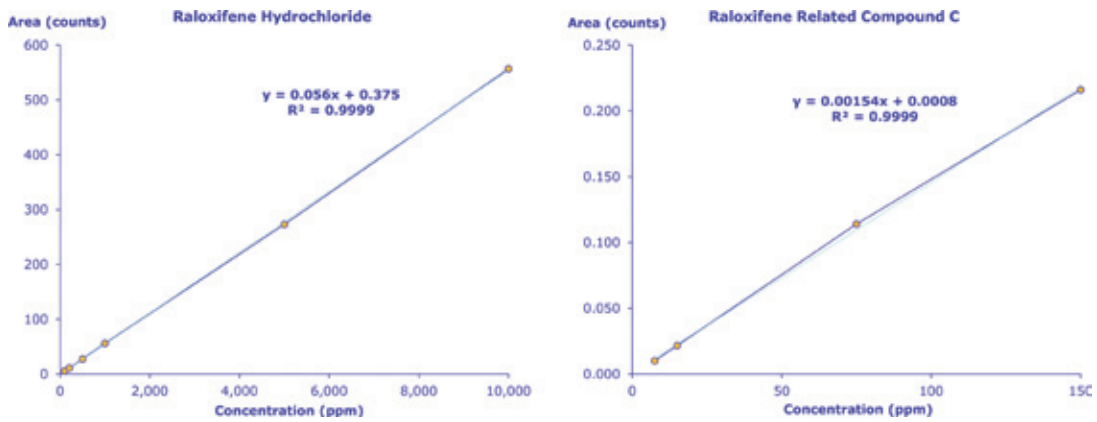
**Pressure drop:** 81–145 Bar (1,175–2,103 psi)

| Time (min) | Solution A (%) | Solution B (%) |
|---|---|---|
| 0 | 75 | 25 |
| 9 | 75 | 25 |
| 40.25 | 50 | 50 |
| 42.25 | 75 | 25 |
| 49 | 75 | 25 |

### Suitability Requirements

- **Resolution:** NLT 2.0 between raloxifene and raloxifene related compound C
- **Tailing factor:** NMT 2.0 for raloxifene

| | Raloxifene HCl (ppm) | Area (counts) | Raloxifene related compound C (ppm) | Area (counts) |
|---|---|---|---|---|
| | 100 | 5.599 | 7.5 | 0.010 |
| | 200 | 10.812 | 15 | 0.022 |
| | 500 | 27.328 | 75 | 0.114 |
| | 1,000 | 55.919 | 150 | 0.216 |
| | 5,000 | 273.231 | | |
| | 10,000 | 556.720 | | |
| LOD | 22 | | 0.18 | 0.25 |
| LOQ | 67 | | 0.54 | 0.75 |




# Assay and Related Substances Validation Data

## Chromatographic Conditions 3

### 1. Specificity

Determined by injection of *System suitability solution* and determination of the retention time and relative retention time for raloxifene hydrochloride and raloxifene related compound C using a Purospher® STAR RP-8 endcapped (5 µm) 250 x 4.6 mm column

| Compound | Retention time (min) | RRT | Tailing factor | Resolution |
|---|---|---|---|---|
| Raloxifene hydrochloride | 17.3 | 0.89 | – | 1.09 |
| Raloxifene related compound C | 19.9 | 1.0 | 6.09 | 1.12 |

### 2. Linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Determined by injecting six concentration levels from 100 to 10,000 ppm of raloxifene hydrochloride and four concentration levels ranging from 7.5 to 150 ppm of raloxifene related compound C

# Recommended Products

## Identification—FTIR <197K>

- Potassium bromide for IR spectroscopy—Uvasol® (Catalogue Number 1.0 4907)

## Assay (HPLC and UHPLC) and Related Substances (HPLC)

- Purospher® STAR RP-8 endcapped (3 µm) 150 x 4.6 mm for assay (Catalogue Number 1.50009.7220)
- Purospher® STAR RP-8 endcapped (2 µm) 100 x 2.1 mm (Catalogue Number 1.50629.0001)
- Purospher® STAR RP-8 endcapped (5 µm) 250 x 4.6 mm for RS analysis (Catalogue Number 1.51454.0001)
- Potassium dihydrogen phosphate for analysis (<= 0.005% Na)—EMSURE® ACS, ISO, Reag. Ph. Eur. (Catalogue Number 1.04877)
- Water for chromatography (LC-MS grade)—LiChrosolv® (Catalogue Number 1.15333) or fresh water from the Milli-Q® system
- Ortho-phosphoric acid 85% for analysis—EMSURE® ACS, ISO, Reag. Ph. Eur. (Catalogue Number 1.00573)
- Potassium hydroxide solution 47% for analysis—EMSURE® (Catalogue Number 1.05545)
- Acetonitrile (isocratic grade for liquid chromatography)—LiChrosolv® Reag. Ph. Eur. (Catalogue Number 1.14291)

## End Notes

[1]Solution A and acetonitrile (70:30)
[2]Tetrahydrofuran and methanol (70:30)