IJPRA Journal

International Journal of Pharmaceutical research and Applications
Volume 11, Issue 2, Mar-Apr 2026, pp:428-435 www.ijprajournal.com ISSN: 2456-4494

Method Development and Validation of RP-HPLC For the Estimation of Myo-Inositol Apis: A Comprehensive Review

Kiran Sharma[1], Dhiraj Kumar[2]

[1] *Student, Department of Pharmaceutical chemistry, Institute of Technology and Management, Gida, Gorakhpur273209*

[2] *Faculty of Pharmacy, Department of Pharmaceutical chemistry, Institute of Technology and Management, Gida, Gorakhpur273209*

Date of Submission: 12-03-2026 Date of Acceptance: 25-03-2026

ABSTRACT: Myo-inositol is an important cyclitol widely used in pharmaceutical formulations, nutraceuticals, and therapeutic preparations, particularly in the management of metabolic disorders such as polycystic ovary syndrome and insulin resistance. Accurate quantification of myo-inositol in bulk drug substances and pharmaceutical dosage forms is essential for quality control and regulatory compliance. Among the available analytical techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as a reliable and widely used method due to its sensitivity, reproducibility, and suitability for routine analysis. This review summarizes recent developments in RP-HPLC methods for the determination of myo-inositol, focusing on chromatographicconditions, mobile phase optimization, detection strategies, and validation parameters according to International Council for Harmonisation (ICH) guidelines. Emphasis is placed on critical validation parameters including linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability. Several studies report the successful application of C18columns with optimized mobile phases consisting of aqueous buffers and organic solvents such as acetonitrile or methanol, enabling efficient separation and accurate quantification of myo-inositol in bulk drugs and combined dosage forms. The review highlights current analytical challenges related to the highly polar nature and weak UV absorbance of myo-inositol and discusses strategies to improve sensitivity and selectivity. Overall, RP-HPLC remains a practical and robust analytical technique for the determination of myo-inositol, supporting pharmaceutical quality control and research applications.

KEYWORDS: Myo-inositol, RP-HPLC, method development, validation, ICH guidelines, API estimation, pharmaceutical analysis.

I. INTRODUCTION

Drug Profile of Myo-Inositol:

Myoinositolisa naturally occurring cyclohexanehexol and the most biologically active isomer of inositol. It is commonly called vitamin B8, but it is not truly an essential vitamin because the human body can produce it on its own.

Myo-inositol is a 6-carbon cyclic polyalcohol that occurs as anhydrous, hydroscopic crystals. Myoinositol has a sweet taste, is soluble in water, and is slightly soluble in alcohol. It is insoluble in ether and other organic solvents.[1] Inositol are pseudo vitamin compoundsthat are falsely said to belong to the B-complex.

PHYSICOCHEMICALPROPERTIES OF MYO-INOSITOL

Myo-inositol is highly polar and freely soluble in water due to the presence of six hydroxyl groups. It lacks a strong chromophoric group, resulting in weak UV absorbance. These properties pose challenges during chromatographic analysis, particularly in reverse phase systems where polar compounds show limited retention. Understandingthese characteristics is essential for selecting appropriate chromatographicconditions and detection methods.

Property of Myo-Inositol API

Chemical Name: Cyclohexane-1,2,3,4,5,6-hexol
Molecular Formula: $C_6H_{12}O_6$
Molecular Weight: 180.16 g/mol
Melting Point: 225–230°C
Solubility: Freely soluble in water

High Performance Liquid Chromatography

HPLC works based on separating components interactions with a stationary phase as they get carried by a mobile phase. Small particle size of stationary phase which gives high surface area to make separation more specific and precise. The use of micro syringes allows samples to be injected into pumps that provide high pressure flow of the mobile phase.[4]When small volume of analyte is injected into column the components will move with different affinity in column and separate out with different retention time and on recorder will give distinct and resolved peaks which is used in analysis of analyte.[5]

Classification of HPLC can be done as

HPLC is often divided into two subclasses according on the mode of operation:[6]

NP- HPLC

The term "Normal Phase High Performance Liquid Chromatography" (NP-HPLC) refers to methods where the mobile phase is less polar than the stationary phase. In NP- HPLC SiO_2, NH_2, -CN, NO_2, ALO3 and diol are used as stationary phase and cyclohexane [7-8].

RP-HPLC

It means Reverse Phase High Performance Liquid Chromatography. In RP-HPLC mobile phase used is polar or slightly polar, but the stationary phase is nonpolar. Separation is primarily based on hydrophobic interactions [9].

Non-polar analytes in the polar mobile phase are attracted to and interact effectively with non-polar SP (stationary phase), leading to longer retention. Polar analytes have weak interactions with the SP and elute quickly as they are more soluble in the polar MP. [10]

The common parts of HPLC instrumentation are

1. Solvent reservoir
2. Pump
3. Sample injector
4. Column
5. Detector

Principle of RP-HPLC:

RP-HPLC is based on hydrophobic interactionsbetween analytes and a non-polar stationary phase, typically C18 or C8 bonded silica columns. The mobile phase is polar, usually consisting of water combined with organic solvents such as methanol oracetonitrile. Separation occurs due to differential partitioning of analytes between the stationary and mobile phases. For polar molecules like myo-inositol, optimization of mobile phase composition and flow conditions is necessary to achieve acceptable retention and peak symmetry.

RP-HPLC Method Development Strategies

Most reported methods employ C18 columns with aqueous-rich mobile phases such as water–methanol or water–acetonitrile systems. Detection is commonly performed at low UV wavelengths (190–210 nm) or using refractive index detection.

System Suitability Parameters for RP-HPLC

A crucial component of the liquid chromatographic approach is a system suitability test. They are used in analysis to make sure that the chromatographic system has adequate resolution and reproducibility. The test's foundation is the idea that the apparatus, electronics, investigative process, and tester under analysis form a single, integrated system that should be assessed as such.[11] System performance before or during analysis is confirm by determining the parameters such as resolution, plate count, reproducibility and tailing factor.[12]

(A)Retention time (RT)
(B)Theoretical plates(N)
(C)Resolution
(D)Tailing factor
(E)Selectivity (α)

Analytical method development using

RP-HPLC-Analytical techniques are always being created, refined, verified, jointly researched, and used. These created techniques are subsequently compiled in sizable compendia like USP, BP and IP, among others. Usually, it just takes a few attempts to get the necessary separation. Typically, method development entails choosing the method

International Journal of Pharmaceutical research and Applications
Volume 11, Issue 2, Mar-Apr 2026, pp:428-435 www.ijprajournal.com ISSN: 2456-4494

requirements and determining the kind of instrumentation to use and why. During the HPLC method development phase, for columns, and mobile phase all be selected when creating n new HPLC techniques. This is because development involvesconsideration of allcharacteristics related to any method. There areseveral steps in the analytical strategy for developing an HPLC method [13-14].

II. Analytical Challenges in Estimation of Myo-Inositol

2.1 High Polarity
The highly hydrophilic nature of myo-inositol results in poor retention on conventional C18 columns when typical organic-rich mobile phases are used.[15]

2.2 Lack of Strong Chromophore
Myo-inositol does not possess conjugated double bonds, leading to weak UV absorption and detection primarily at low wavelengths (200–210 nm), which may increase baseline noise.[16]

2.3 High Aqueous Solubility
While advantageous for sample preparation, high solubility demands careful mobile phase optimization to avoid early elution and peak distortion.[17]

III. RP-HPLC Method Development Strategy

3.1 Selection of Stationary Phase
C18 columns (250 mm × 4.6 mm, 5 µm) are commonly preferred due to:

. Wide availability

. High theoretical plate count

. Reproducibility

. Compatibility with aqueous mobile phases

Polar-embedded phases may also improve retention but are less commonly available in routine QC settings. [18-20]

3.2 Mobile Phase Optimization
Effective retention is achieved using a highly aqueous mobile phase.

Optimized Conditions Reported in Literature

. Water: Acetonitrile (85:15 v/v) [21]

. pH adjusted to 3.0 with orthophosphoric acid

High aqueous content enhances retention, while slight acidification improves peak symmetry and reproducibility. [22-23]

3.3 Detection Wavelength Selection
UV spectral scanning of analytes typically shows maximum absorbance in the deep-UV region (~200–210 nm), corresponding to high-energy transitions of common organic chromophores such as $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions [24].

For reversed-phase HPLC with UV detection, detection at ~210 nm is widely adopted because it balances sensitivity (by capturing strong absorbance near λ.max for many small molecules) and acceptable baseline stability for typical mobile phases such as water/acetonitrile. Although lower wavelength (<200 nm) can offer increased analyte absorbance, solvent and mobile-phase background absorbance and detector noise often increase significantly below ~210 nm, degrading baseline stability and signal-to-noise performance.[25-26]

Parameter	Typical Condition
Column	C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Water: ACN (85:15), pH 3.0
Flow Rate	1.0 mL/min
Detection	210 nm
Injection Volume	20 µL
Retention Time	~5 min
Run Time	10 min

IV. Method Validation as per ICH Q2(R1)
For the estimation of Myo-Inositol by Reverse Phase High Performance Liquid Chromatography, the analytical method must be validated according to guidelines such as ICH Q2(R1). Validation ensures reliability and reproducibility of the developed analytical method.[34]

4.1 Specificity
Specificity evaluates whether the method can accurately measure myo-inositol in the presence of impurities, degradation products, or excipients.[35]

Evaluation

Inject blank, standard, and samplesolutions

. Confirm no interference at the retention time of myo-inositol.

Acceptance Criteria

. Peak purity should be acceptable.

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. No overlapping peaks at the analyte retention time. [36-37]

4.2 Linearity

Linearity determines whether the analytical response is directly proportional to the concentration of myo-inositol.[38]

Procedure

- Prepare standard solutions at different concentrations (e.g., 10–100 µg/mL).
- Inject each concentration into the RP-HPLC system

Evaluation

- Plot Peak Area vs Concentration.

Acceptance Criteria

- Correlation coefficient (**R^2**) ≥ 0.999.

4.3 Accuracy

Accuracy indicates how close the measured value is to the true value.

Procedure

- Perform **recovery studies** at three levels:
  - 80%
  - 100%
  - 120%

Acceptance Criteria

- % Recovery between 98–102%.[39]

4.4 Precision

Precision measures the reproducibility of the method.[40]

(a) Repeatability (Intra-day Precision)

- Analyze the same sample multiple times on the same day.

(b) Intermediate Precision (Inter-day Precision)

- Analyze the sample on different days or by different analysts.

Acceptance Criteria

- %RSD ≤ **2%**.

4.5 Limit of Detection (LOD)

The lowest amount of myo-inositol that can be detected but not necessarily quantified.[41]

Formula

$$LOD = \frac{3.3 \times \sigma}{S}$$

Where
σ = Standard deviation of response
S = Slope of calibration curve

4.6 Limit of Quantification (LOQ)

The lowest concentration that can be quantitatively measured with acceptable precision.

Formula

$$LOQ = \frac{10 \times \sigma}{S}$$

4.7 Robustness

Robustness evaluates the effect of small changes in chromatographic conditions.

Examples of Changes

- Flow rate ±0.1 mL/min
- Mobile phase composition ±2%
- Detection wavelength ±2 nm
- Column temperature variation

Acceptance Criteria

- %RSD within acceptable limits (<2%).

4.8 System Suitability

System suitability ensures the HPLC system is performing properly before analysis.[42]

Typical Parameters

- Retention time
- Theoretical plates
- Tailing factor
- Peak area reproducibility

Acceptance Criteria

%RSD of peak area ≤ 2%

4.9 Range [43]

The range is the interval between the highest and lowest concentration levels where the method shows acceptable accuracy, precision, and linearity.
Example: 10–100 µg/mL.

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V. Comparison with Alternative Techniques

Technique	Advantages	Limitations
RP-HPLC	Widely available, cost-effective, simple	Weak UV absorbance
HILIC	Better retention for polar compounds	Less common in QC labs
Derivatization-based HPLC	Improved sensitivity	Time-consuming, complex
Ion Chromatography	High selectivity	Specialized equipment

RP-HPLC without derivatization remains the mostpractical approach for routine bulk API analysis.
[44-48]

VI. Applications in Pharmaceutical Quality Control

The validated RP-HPLC method is suitable for:

- Assay of bulk API
- Content uniformity testing
- Stability studies
- Process validation samples

The method's simplicity and compliance with regulatory validation parameters make it ideal for routine pharmaceutical analysis. [49-50]

VII. Future Perspectives

Future analytical advancements may include: [51-52]

- Use of ultra-performance liquid chromatography (UPLC) for reduced run time
- Coupling with mass spectrometry for enhanced sensitivity
- Green analytical chemistry approaches using eco-friendly solvent

VIII. Conclusion

RP-HPLC remains a reliable and practical analytical technique for estimation of myo-inositol API in bulk drug substances. Despite analytical challenges associated with high polarity and weak UV absorption, careful optimization of mobile phase composition and detection wavelength enables accurate and precise quantification. Validation as per ICH Q2(R1) guidelines confirms the suitability of the method for routine quality control applications. Continued methodological refinement may further enhance sensitivity, efficiency, and environmental sustainability. [53-56]

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