Chromai — From Ingredient Labeling to Content Determination: Comprehensive Safeguarding of Medication Safety for Pharmaceutical Lactose

Determination of Lactose in Pharmaceutical Excipients by HPLC with Refractive Index Detector (RID)

1. Introduction

Lactose is a widely used pharmaceutical excipient that serves as a filler, diluent, binder, glidant, lubricant, stabilizer, and sweetener. It is broadly applied in the manufacture of tablets, capsules, granules, and oral liquids. Although lactose is generally regarded as safe, lactose-intolerant patients must exercise caution when using lactose-containing medications, as consumption may trigger symptoms such as bloating and diarrhea. For this reason, lactose, as a mandatory excipient, must be clearly stated in the product ingredient list (including packaging and package inserts).

This application note references the lactose testing method described in the Pharmaceutical Excipients section of the Chinese Pharmacopoeia (2025 Edition, Volume IV). A Chromai Ultra-High Performance Liquid Chromatograph (UHPLC) equipped with an amino column for separation and a Refractive Index Detector (RID) for detection was employed to accurately determine the lactose content in pharmaceutical excipients.

2. Experimental

2.1 Instrumentation

Leaps Ultra-High Performance Liquid Chromatograph (UHPLC) system, comprising:

- P40 Quaternary Gradient Pump
- A10C Autosampler
- C10 Column Oven
- D60L Refractive Index Detector (RID)

2.2 Solution Preparation

(1) Lactose Standard Stock Solution (20 mg/mL)

Accurately weigh an appropriate quantity of lactose reference standard. Dissolve in water and dilute to prepare a solution containing 20 mg per 1 mL.

(2) Sucrose Standard Stock Solution (20 mg/mL)

Accurately weigh an appropriate quantity of sucrose reference standard. Dissolve in water and dilute to prepare a solution containing 20 mg per 1 mL.

(3) System Suitability Solution (5 mg/mL each)

Transfer 2.5 mL each of the lactose and sucrose standard stock solutions into a 10 mL volumetric flask. Dilute with water to volume and mix thoroughly.

(4) Lactose Standard Calibration Solutions

Dilute appropriate volumes of the lactose standard stock solution with purified water to prepare calibration solutions at the following concentrations: 0.2 mg/mL, 2.0 mg/mL, 4.0 mg/mL, 6.0 mg/mL, and 10.0 mg/mL.

2.3 Chromatographic Conditions

Mobile phase: Acetonitrile : Water = 70 : 30 (v/v)

Flow rate: 1.0 mL/min, isocratic elution

Column: Lotus NH_2, 4.6 mm × 250 mm, 5 µm

Injection volume: 10 µL

Column temperature: 30 °C

RID temperature: 30 °C

3. Results and Discussion

3.1 Standard Solution Chromatogram

Figure 1 shows the chromatogram of the lactose standard solution. The peak is sharp and symmetrical, indicating excellent separation performance.

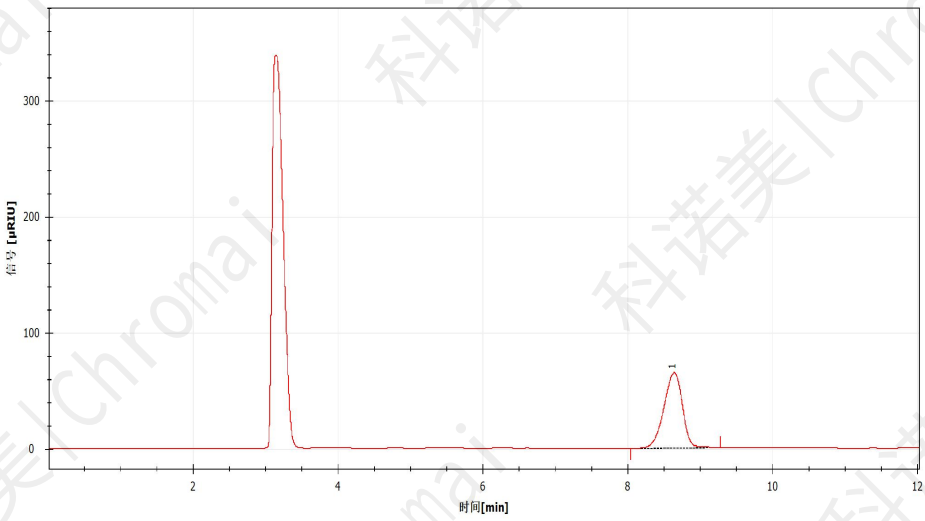

Figure 1 Chromatogram of Lactose Standard Solution (Peak 1: Lactose)

3.2 System Suitability

Figure 2 shows the system suitability chromatogram containing both sucrose and lactose. The resolution between the two compounds was 3.2, meeting the requirements specified in the Chinese Pharmacopoeia (resolution ≥ 1.5).

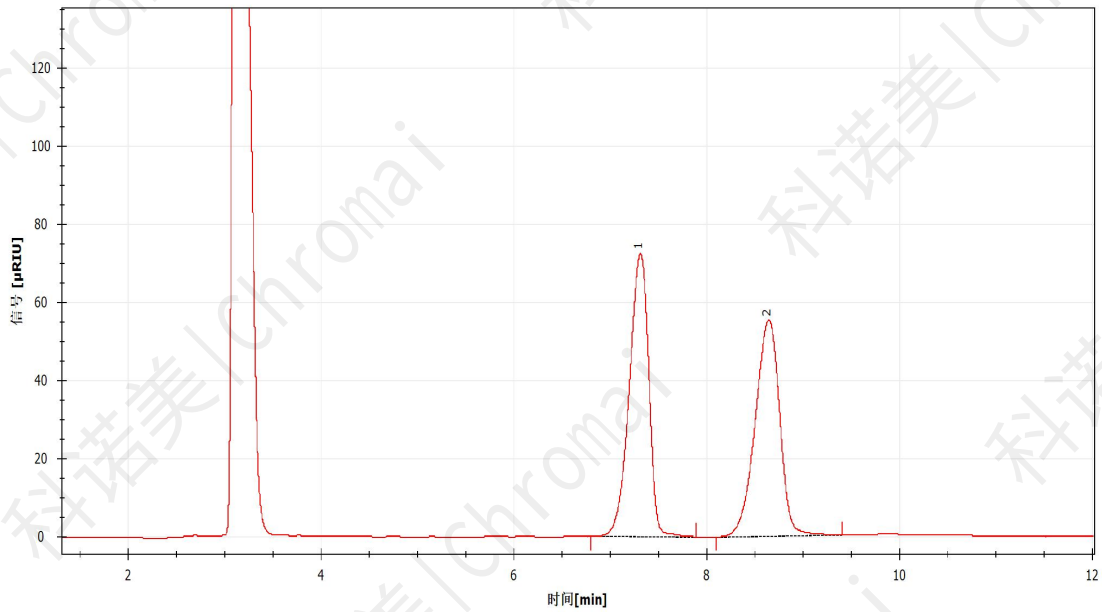

Figure 2 System Suitability Chromatogram (Peak 1: Sucrose; Peak 2: Lactose)

3.3 Linearity and Limit of Detection (LOD)

Standard solutions at five concentration levels (0.20, 2.0, 4.0, 6.0, and 10.0 mg/mL) were injected. The peak area vs. concentration data were subjected to linear regression. The method demonstrated excellent linearity over the tested range.

Table 1 Linearity and Detection Limit of Lactose

Compound	Linear Range (mg/mL)	Linear Equation	Correlation Coefficient (R)	LOD (mg/mL)
Lactose	0.20 ~ 10.0	y = 192.94x – 2.1977	0.9999	0.016

3.4 Repeatability

The lactose standard solution at 10.0 mg/mL was injected six consecutive times under identical chromatographic conditions. The relative standard deviations (RSD) of retention time and peak area were calculated to assess method repeatability.

Table 2 Repeatability of the Method

Compound	Retention Time RSD (%)	Peak Area RSD (%)
Lactose	0.06	0.26

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4. Conclusion

This method was established with reference to the lactose testing requirements in the Chinese Pharmacopoeia (2025 Edition, Volume IV — Pharmaceutical Excipients). Using the Chromai Leaps UHPLC system equipped with an amino column and a Refractive Index Detector, the method demonstrated the following key performance characteristics:

- Excellent system suitability: resolution between sucrose and lactose = 3.2 (≥ 1.5, compliant with pharmacopoeia)
- Outstanding linearity: R = 0.9999 over the range 0.20 ~ 10.0 mg/mL
- High sensitivity: limit of detection = 0.016 mg/mL
- Superior repeatability: retention time RSD = 0.06%; peak area RSD = 0.26%

The method is stable, sensitive, and provides excellent separation, making it fully suitable for the accurate determination of lactose content in pharmaceutical excipients.

Reference: Chinese Pharmacopoeia 2025 Edition, Volume IV — Pharmaceutical Excipients (Lactose)

Instrument: Chromai Leaps UHPLC System | Column: Lotus NH_2 4.6×250 mm, 5 μm