



National Standard of the Peoples Republic of China

GB/T -

Determination of the activity of heparin lyase – Spectrophotometric method

肝素酶活力的测定 分光光度法

(English Translation)

(报批稿)

Issue date

Implementation date

Issued by General Administration of Quality Supervision,
Inspection and Quarantine of the People's Republic of China
and standardization administration

Foreword

This standard is drafted in accordance with the rules given in the GB/T 1.1-2009 *Directives for standardization-Part 1: Structure and drafting of standards*.

This standard was prepared by SAC/TC 387 (National Technical Committee 387 on Biochemistry Products and Testing Technology of Standardization Administration of China) .

National Institute of Measurement and Testing Technology is in charge of this English translation. In case of any doubt about the contents of English translation, the Chinese original shall be considered authoritative.

Determination of the activity of heparin lyase - Spectrophotometric method

1 Scope

This document describes a spectrophotometric method for the determination of heparinase activity.

This document applies to the determination of the biochemical reagents Heparinase I, II and III activities.

2 Normative references

The contents of the following documents constitute indispensable provisions of this document through normative references in the text. Where a document is cited with a date, only the version corresponding to that date applies to this document; where a document is cited without a date, the latest version (including all change orders) applies to this document.

GB/T 6682 specification and test methods for water for analytical laboratories.

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 Heparin lyase activity unit

The amount of enzyme required to produce 1 μmol of 4,5-unsaturated glucuronide per minute of enzymatic hydrolysis at 30 $^{\circ}\text{C}$ and pH 7.0 was determined using 1,000 μL of heparin at a concentration of 8.3 g/L (heparinase I and II) or 1,000 μL of acetylheparin sulfate at a concentration of 16.7 g/L (heparinase III) as substrates.

Note: Enzyme activity units are expressed in U.

4 Principle

Heparinase catalyzes the cleavage of the substrate heparin or acetylheparin sulfate to produce the cleavage product 4,5-unsaturated glucuronide. 4,5-unsaturated glucuronide has a characteristic absorption peak at 232 nm. The enzyme activity was calculated by measuring the change in absorbance value at 232 nm in combination with the $A_{232\text{ nm}}$ molar extinction coefficient of 4,5-unsaturated glucuronic acid.

5 Reagents and materials

Unless otherwise specified, the reagents used in this method are analytically pure, and the water is secondary water as specified in GB/T 6682.

5.1 reagents

5.1.1 Heparin sodium (CAS No. 9041-08-1): purity $\geq 99\%$, or standardized product certified by the state and granted with certificate.

5.1.2 Acetylheparin Sulfate (CAS No. 9050-30-0): purity $\geq 99\%$, or standardized product certified by the state and granted with certificate.

5.1.3 Hydrochloric acid (HCl)

5.1.4 Tris(hydroxymethyl)aminomethane (Tris)

5.1.5 Calcium chloride (CaCl_2)

5.1.6 Sodium chloride (NaCl)

5.2 Reagent Configuration

5.2.1 Hydrochloric acid solution (6 mol/L)

Accurately measure 50 mL of hydrochloric acid (5.1.3) in a 100 mL volumetric flask, dilute with water to a constant volume, and shake well.

5.2.2 Heparinase I reaction substrate solution

Accurately weigh Tris (5.1.4) 0.121 g, CaCl_2 (5.1.5) 0.0067 g, NaCl (5.1.6) 0.584 g, and heparin sodium (5.1.1) 0.415 g. Dissolve in water and transfer to a 50 mL volumetric flask, adjust the pH to 7.0 with hydrochloric acid solution (5.2.1), dilute and set the volume with water to the scale and mix well.

5.2.3 Heparinase II reaction substrate solution

Accurately weigh Tris (5.1.4) 0.121 g, CaCl_2 (5.1.5) 0.0067 g, NaCl (5.1.6) 0.292 g, heparin sodium (5.1.1) 0.415 g, dissolve with water and transfer to a 50 mL volumetric flask, adjust pH to 7.0 with hydrochloric acid solution (5.2.1), dilute and set the volume with water to the scale and mix well.

5.2.4 Heparinase III reaction substrate solution

Accurately weigh Tris (5.1.4) 0.121 g, CaCl_2 (5.1.5) 0.0067 g, NaCl (5.1.6) 0.146 g, and Acetylheparin Sulfate (5.1.2) 0.835 g. Dissolve in water and transfer to a 50 mL volumetric flask, adjust the pH with Hydrochloric Acid Solution (5.2.1) to 7.0, and dilute to the scale with water. Mix well.

6 Instruments and equipment

6.1 UV-Vis spectrophotometer: with temperature-controlled cuvette tank, wavelength accuracy of 1 nm, absorbance value accuracy of 0.001.

6.2 Analytical balance: 1 mg and 0.1 mg sensitivities, respectively.

6.3 pH meter: accuracy 0.01.

6.4 Magnetic stirrer.

- 6.5 Quartz cuvette: with lid, 1 cm.
- 6.6 Volumetric flasks: 50 mL, 100 mL.
- 6.7 Beakers: 50 mL, 1,000 mL.
- 6.8 Measuring cylinder: 50 mL.
- 6.9 Pipette: 10 μ L, 100 μ L, 1 000 μ L.

7 Testing procedures

7.1 Sample Preparation

According to the expressed enzyme activity of the samples, pipette a liquid sample of heparinase or weigh an appropriate amount of a solid sample of heparinase (accurate to 0.000 1 g), dissolve it in water and dilute it to between 1.5~9 U/mL, and leave it to be measured.

7.2 Assay

Accurately pipetted 1 000 μ L of reaction substrate solution in a quartz cuvette (6.5), placed in the temperature-controlled cuvette slot of the spectrophotometer, and after the temperature was stabilized at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 10 μ L of the solution to be measured was quickly added, and then put into the spectrophotometer immediately after mixing with a lid upside down, and then scanned for 20 min at 232 nm, and the absorbance curves were saved.

8 Data processing

8.1 Slope

The first 60 s segment where the curve was smooth and linear was selected and the slope was calculated by a linear regression equation.

$$k = \frac{A_1 - A_0}{t \times 60} \dots \dots \dots (1)$$

Style:

k - slope of the absorbance curve in absorbance per minute (Abs/min);

A_0 - the initial absorbance value of the selected zone;

A_1 - the final absorbance value of the selected zone;

t - reaction time in minutes (min).

The results of the calculations are retained to three significant figures.

8.2 Enzyme activity

8.2.1 Liquid samples

Heparinase activity in the specimen was calculated according to equation (1):

$$X_1 = \frac{(V_1 + V_2) \times k \times D}{\epsilon \times V_2} \dots \dots \dots (2)$$

Style:

X_1 - enzyme activity in units of enzyme activity per milliliter (U/mL)

V_1 - Volume of reaction substrate solution in microliters (μL);

V_2 - volume of liquid to be measured in microliters (μL);

k - slope of the absorbance curve in absorbance per minute (Abs/min);

D - dilution factor;

ε - molar extinction coefficient of 4,5-unsaturated glyoxalate at $A_{(232)\text{ nm}}$, $3.8\text{ mM}^{-1}\text{ cm}^{-1}$.

The results of the calculations are retained to three significant figures.

8.2.2 Solid Samples

Heparinase activity in the specimen was calculated according to equation (2):

$$X_2 = \frac{(V_3 + V_4) \times k}{\varepsilon \times V_4} \times \frac{V_0}{m} \dots\dots\dots (3)$$

Style:

X_2 - enzyme activity in units of enzyme activity per gram (U/g)

V_3 - Volume of reaction substrate solution in microliters (μL);

V_4 - volume of liquid to be measured in microliters (μL);

k - slope of the absorbance curve in absorbance per minute (Abs/min);

ε - Molar extinction coefficient of 4,5-unsaturated glucuronic acid at $A_{232\text{ nm}}$, $3.8\text{ mM}^{-1}\text{ cm}^{-1}$. Units are milliliters (mL);

V_0 - the volume of the solution to be measured in milliliters (mL);

m - sample mass in grams (g).

The results of the calculations are retained to three significant figures.

9 Precision

Under conditions of repeatability, the absolute difference between two independent determinations obtained should not exceed 10% of the arithmetic mean.
