



4. Assay

4.1 Reagents and solutions

Control glucuronic acid product

Borax sulfuric solution: weigh 9.54g sodium tetraborate and dissolve it into 1000mL concentrated sulfuric acid.

Carbazole solution: weigh 0.125g carbazole precisely, dissolve it by absolute ethyl alcohol, reach the constant volume in a 100mL measuring flask and store it in a brown bottle.

Standard glucuronic acid storage solution: weigh 50mg standard glucuronic acid product which has been dried in vacuum to constant weight under 105℃, place it into a 100mL measuring flask, add water to dissolve and dilute the solution to scale, and shake the solution even.

Preparation of standard glucuronic acid solution: use pipette tube to absorb 10mL standard glucuronic acid storage solution into a 100mL measuring flask. Then, add purified water to dilute the solution to scale to obtain the 50 μg/mL standard glucuronic acid solution.

4.2 Preparation of test solution

The sample concentration required by the concentration inspection is 0.1% (referring to the mass ratio after the moisture content is removed). According to the measured result of loss on drying of test sample (h), calculate the weight W1 of needed sample based on the calculation formula $W1 = 0.1 * 100 / (100 - h)$. The weighing and measurement shall be precise to 1mg. Place the weighed sample into a 100mL measuring flask, add 50~70mL purified water, shake them to make the sample suspended into the solution, and then place the solution into water shaking bath under 50℃ in order to make the sample fully dissolved. After the solution is even, take the solution out and cool it to room temperature. Use purified water to dilute the solution to scale, and move the solution into the beaker after mixing the solution up evenly. Weigh about 5.0g (W2) (the density is about 1.00g/mL, equivalent to 5.0mL), place it into a 50mL measuring flask, add water to dilute the sample to scale and shake the solution even to obtain the test solution.

4.3 Preparation of standard curve

Precisely weigh 0, 0.2, 0.4, 0.6, 0.8 and 1.0mL of the standard glucuronic acid solution, respectively place them into 25mL test tubes with stopper, and then add water to make the total volume to 1.0mL. Mix it evenly and cool it in ice bath, then slowly add 5.0mL borax sulfuric acid solution which has been cooled in ice bath. Then, enclose them, mix them evenly, heat them in boiling water bath for 10mins and cool them to room



temperature in ice bath. Add 0.2ml carbazole solution into the test tubes and mix it evenly. enclose the tubes and heat them in boiling water bath for 15mins. Then cooled them in the ice bath.

Use T6 New Century ultraviolet-visible spectrophotometer to measure the absorbance at the wave band with a length of 530nm. Afterwards, calculate the regressive equation for corresponding absorbance based on the concentration of glucuronic acid (the regressive correlation coefficient $r > 0.990$).

4.4 Measurement of test solution

Take four 25mL colorimetric tubes and add 1mL test solution into each of them. Then, take another 25mL colorimetric tube and add 1mL purified water into it to serve as the blank sample. Operate according to Article 2.3 from the part of “mix it evenly and cool it in ice bath”.

Use T6 New Century ultraviolet-visible spectrophotometer to measure the absorbance at the wave band with a length of 530nm. Put the measured absorbance into the regressive equation to calculate the concentration of glucuronic acid C1 in test solution.

4.5 Calculation:

The final result shall be the average and RSD shall not be higher than 5%.

Calculate the content of glucuronic acid XG and the concentration of sodium hyaluronate XHA respectively according to formula (1) and (2):

$$X_G = \frac{C \times 50 \times 100 \times 100 \times 10^{-6}}{W_1 \times (100 - h) \times W_2} \times 100\% \dots \dots \dots (1)$$

$$X_{HA} = \frac{C \times 50 \times 100 \times 100 \times 10^{-6} \times 401.3}{W_1 \times (100 - h) \times W_2 \times 194.1} \times 100\% \dots \dots \dots (2)$$

Where,

C1-concentration of glucuronic acid in test solution calculated by regressive equation, μ g/mL;

W1-weight of test sample, g;

W2-weight of diluted test solution in the second step, g;

h - loss on drying, %;

401.3-molecular weight of sodium hyaluronate in disaccharide unit;