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Determination of reducing sugar in biochemical products - High-performance liquid chromatography with pre-column derivatization

生化制品中还原糖的测定 柱前衍生高效液相色谱法

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Table of Contents

Determination of reducing sugar in biochemical products - High-performance liquid chromatography with pre-column derivatization

1 Scope

This document specifies the determination method that uses high-performance liquid chromatography with pre-column derivatization to determine 10 reducing sugars: D-mannose, D-ribose, L-rhamnose, D-galactose, L-arabinose, D-xylose, L-fucose, D-glucose, D-maltose, D-lactose, in biochemical products.

This document is applicable to the determination of mannose, ribose, rhamnose, galactose, arabinose, xylose, fucose, glucose, maltose, lactose content in glycopepsin, human immune globulin for intravenous injection, rabies immunoglobulin, modified Y medium, glucose broth medium, buffer power-nitrate, xylose-gelatin medium and other biochemical products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682, *Water for analytical laboratory use - Specification and test methods*

3 Terms and definitions

There are no terms and definitions that need to be defined in this document.

4 Principle

The reducing sugars in the sample are extracted by boiling water. The extract is reacted with 1-phenyl-3-methyl-5-pyrazolone (PMP) under alkaline conditions to generate sugar derivatives. Separate by liquid chromatography and determine by UV detector. Determine the nature by chromatographic retention time. Use external standard method for quantification.

5 Reagents or materials

Unless otherwise specified, all reagents used in this method are analytically pure except that the mobile phase is chromatographically pure. The water used in the test is grade one water specified in GB/T 6682.

5.1 Potassium dihydrogen phosphate (KH_2PO_4).

5.2 Sodium hydroxide (NaOH).

5.3 Potassium ferrocyanide [$K_4Fe(CN)_6 \cdot 3H_2O$]

5.4 Zinc acetate [$Zn(CH_3COO)_2 \cdot 2H_2O$]

5.5 1-Phenyl-3-methyl-5-pyrazolone ($C_{10}H_{10}N_2O$, PMP).

5.6 Hydrochloric acid (HCl).

5.7 Trichloromethane ($CHCl_3$).

5.8 Methanol (CH_3OH).

5.9 Acetonitrile (CH_3CN): Chromatographically pure.

5.10 Triethylamine ($C_6H_{15}N$).

5.11 N-hexane (C_6H_6).

5.12 Potassium ferrocyanide solution (106g/L): Weigh 106.0g of potassium ferrocyanide. Use water to dissolve and dilute to 1000mL.

5.13 Zinc acetate solution (220g/L): Weigh 220.0g of zinc acetate. Use water to dilute to 1000mL.

5.14 0.5mol/L 1-phenyl-3-methyl-5-pyrazolone (PMP) derivative solution: Weigh 87.1g of PMP derivatization reagent. Add methanol to dissolve and dilute to 1000mL.

5.15 1.0mol/L NaOH solution: Weigh 40.0g of sodium hydroxide. Add water to dissolve and dilute to 1000mL.

5.16 1.0mol/L HCl solution: Take 83.2mL of concentrated hydrochloric acid. Slowly add into about 800mL of water. After cooling, add water to make up to 1000mL.

5.17 Sugar standard product: D-mannose (CAS:3458-28-4), D-ribose (CAS:50-69-1), L-rhamnose (CAS:10030-85-0), D-galactose (CAS:59-23-4), L-arabinose (CAS: 5328-37-0), D-xylose (CAS: 31178-70-8), L-fucose (CAS: 2438-80-4), D-glucose (CAS:50-99-7), D-maltose (CAS:3458-28-4), D-lactose (CAS:63-42-3). The purity is all ≥99%.

After cooling the sample solution, add 5mL of n-hexane. Vortex for 2min. Centrifuge at 5000r/min for 5min. Discard the n-hexane layer. Pour the aqueous supernatant into a 50mL volumetric flask. Add 10mL of water to the solid residue. Vortex for 2min. Centrifuge at 5000r/min for 3min. Combine the aqueous layer in the above 50mL volumetric flask. Add 5mL of potassium ferrocyanide solution, 5mL of zinc acetate solution. Use water to set volume to constant. Mix well. Centrifuge to get the supernatant. After filtration through a 0.45m filter membrane, it can be used.

7.2.2 Preprocessing of liquid samples containing oil and protein

Weigh 5g of sample (accurate to 0.001g) into a 50mL centrifuge tube. Add 20mL of water. Extract in boiling water bath for 15min. Conduct ultrasonic extraction for 10min. After the sample solution is cooled, add 3mL of potassium ferrocyanide solution and 3mL of zinc acetate solution. Use water to set volume to 40mL. Shake the centrifuge tube well. Centrifuge at 5000r/min for 5min. Take part of the clarified water layer. Add 5mL of n-hexane. Shake or vortex for 2min. Centrifuge at 5000r/min for 5min. Take the clear liquid from the water layer. After filtration through a 0.45μm filter, it can be used.

7.2.3 Preprocessing of liquid samples not containing oil and protein

Weigh 5g of sample (accurate to 0.001g) into a 50mL centrifuge tube. Add water to 40mL. Extract in boiling water bath for 15min. Conduct ultrasonic extraction for 10min. After the sample solution has cooled, transfer to a 50mL volumetric flask to set volume to constant. Take the clear liquid from the water layer. After filtration through a 0.45μm filter, it can be used.

7.3 Specimen derivation

Pipette 0.4mL of specimen and standard working solution of different concentrations into a 15mL stoppered test tube. Add 0.2mL of 1.0mol/L NaOH solution and 1.0mL of 0.5mol/LPMP derivative solution. React in a constant temperature water bath at 70°C for 60min. Cool to room temperature. Add 0.2mL of 1.0mol/L HCl solution. After shaking, use nitrogen to dry. Add 2mL of distilled water to reconstitute. Add 2mL of chloroform. After fully vortexing, stand or centrifuge. Discard the lower organic layer. Repeat this three times. Take the upper aqueous phase. After passing through a 0.45μm filter membrane, it is analyzed by liquid chromatography.

7.4 Instrument chromatographic reference conditions

The instrument chromatographic reference conditions are as follows:

a) Chromatographic column: C_{18} chromatographic column, 250mm×4.6mm, 3.0μm, or a chromatographic column with equivalent performance;

b) Mobile phase: Phase A is an aqueous solution containing 20mmol/LKH_2PO_4 (adjusted to pH 7.8±0.1 with triethylamine). Phase B is acetonitrile. The gradient

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