



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

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**National food safety standards--Determination of lactoferrin in
food**

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Table of Contents

1 Scope	3
2 Principle	3
3 Reagents and materials	3
4 Instruments and equipment	5
5 Analysis procedure	5
6 Expression of analysis results	7
7 Precision	7
8 Others	7
Annex A Liquid chromatogram of bovine lactoferrin standard solutions	8
Annex B Heparin affinity column validation method	9

1 Scope

This Standard specifies the high-performance liquid chromatography method for the determination of lactoferrin in food.

This Standard applies to the determination of bovine lactoferrin content in pasteurized milk, prepared milk, milk-containing beverages, prepared milk powder and milk-based infant formula.

This Standard does not apply to the determination of bovine lactoferrin in flavored fermented milk and soy-based infant formula.

2 Principle

The bovine lactoferrin in the sample is extracted with phosphate buffer, enriched and purified with a heparin affinity column, then separated by a reversed-phase high-performance liquid chromatography column, detected with a UV detector at 280 nm, and quantified by the external standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical reagent, and the water is Grade 1 water specified in GB/T 6682.

3.2.1 Phosphate buffer I. Weigh 7.1 g of disodium hydrogen phosphate and 58.4 g of

sodium chloride, add 900 mL of water to dissolve, adjust the pH to 8.0 $\pm$ 0.2 with phosphoric acid, add water to make the volume to 1000 mL, and freshly prepare each time before use.

3.3 Reference material

Bovine lactoferrin reference material (CAS number. 146897-68-9). purity $\geq$ 95 %, iron content $\leq$ 35 mg/100 g, or a reference material certified by the country and awarded a reference material certificate.

3.4.1 Bovine lactoferrin standard stock solution (5.0 mg/mL). Weigh 50 mg (accurate

to 0.1 mg) of bovine lactoferrin reference material, dissolve it in water, transfer to a 10 mL volumetric flask and dilute to the mark, and mix well. Store at 0 degrees C $\sim$ 4 degrees C, it can be stored for 3 months.

3.5.1 Heparin affinity column. 1 mL, column capacity $\geq$ 2 mg, or one with equivalent

performance (see Annex B for verification method).

4.1 High-performance liquid chromatograph. equipped with a UV detector or equivalent.

5.1.1 Preparation of samples

Take at least 200 g of a representative sample. If it is a liquid sample, shake well and store it in a container as a sample, store it at 0 degrees C $\sim$ 4 degrees C for later use. If it is a solid sample, mix well and store it in a container as a sample, store it dry at room temperature for later use.

5.1.3 Purification of samples

Before use, add 5.0 mL of phosphate buffer II to activate the heparin affinity column.

Accurately pipette 10.0 mL of the above sample solution to pass through the column, control the flow rate at 1 mL/min $\sim$ 3 mL/min.

5.2.1 Chromatographic column. C4, 5 μ m, 300Å, 250 mm x 4.6 mm (inner diameter) or

equivalent.

5.3 Plotting of standard curve

Respectively inject the bovine lactoferrin standard series working solutions into the high-performance liquid chromatograph, determine the corresponding peak areas, and plot a standard curve with the concentrations of bovine lactoferrin in the standard working solutions as the abscissa and the peak areas as the ordinate.

5.4 Determination of sample solution

Inject the sample solution into the high-performance liquid chromatograph to obtain the peak area, then obtain the concentration of bovine lactoferrin in the sample according to the standard curve.

6 Expression of analysis results

The bovine lactoferrin content in the sample is calculated according to formula (1).

7 Precision

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10 % of the arithmetic mean.

8 Others

When the sampling volume of the liquid sample is 5.00 g and the constant volume is 25 mL, the detection limit of the liquid sample in this method is 0.3 mg/100 g and the quantification limit is 1 mg/100 g; when the sampling volume of the solid sample is 5.00 g and the constant volume is 50 mL, the detection limit is 2 mg/100 g and the quantification limit is 6 mg/100 g.

Annex A Liquid chromatogram of bovine lactoferrin standard solutions

The liquid chromatogram of bovine lactoferrin standard solutions (50.0 ug/mL) is shown in Figure A.1.

Figure A.1 -- Liquid chromatogram of bovine lactoferrin standard solutions (50.0 ug/mL)

Annex B Heparin affinity column validation method

B.1 Column capacity verification Take 0.40 mL of the 5.0 mg/mL bovine lactoferrin standard stock solution and pass it through the heparin affinity column instead of the sample solution, operate according to 5.1.3, use a liquid chromatograph to measure the bovine

lactoferrin content and calculate its recovery rate. Test at least 2 heparin affinity columns from the same batch.

B.2 Result judgment If the recovery rate of bovine lactoferrin is $\geq 95\%$, it is a usable product.

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