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**CERTIFICATION AND ACCREDITATION INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

RB/T 033-2020

**Guidance on verification and validation of microbiological
testing methods
(English Translation)**

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1 Scope

This Standard specifies the principle, the used reagents and instruments, the determination procedures, the calculation method of the results, the sensitivity, accuracy and precision of the high-performance liquid chromatography method for the determination of astaxanthin content in fish and fishery products.

This Standard is applicable to the determination of astaxanthin content in fish, crustaceans, shrimp meal, krill oil and other products.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this document.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

GB/T 30891 Practice of Sampling Plans for Aquatic Products

3 Principle

The to-be-tested substance in the sample is extracted by the mixed solution of acetone or dichloromethane-methanol; after alkali saponification, the astaxanthin ester is converted into free astaxanthin; separated by liquid chromatography; measured by ultraviolet detector; and quantified by external standard method.

4 Reagents

4.1

Unless otherwise specified, the used reagents are all analytically pure; and the water is the Class-I water specified in GB/T 6682.

protected from light. The validity period is 1 month.

4.21 Preparation of geometric isomers of astaxanthin: Accurately pipette an

appropriate amount of all-trans astaxanthin standard stock solution (4.20); dilute with acetone to prepare a standard solution of 10 μ g/mL. Pipette 2mL of standard solution into 10mL test tube with a stopper. Add 3mL of dichloromethane; mix well; add 50 μ L of

0.01 g/mL iodine-dichloromethane solution (4.17); vortex thoroughly; seal and place under natural light for reacting 15min. Then add 1mL of 0.1mol/L sodium thiosulfate solution (4.18) to shake sufficiently and remove excessive iodine; then stand and separate into layers to take the lower phases. After drying with nitrogen, add 1mL of acetone to dissolve it; and it shall be prepared for immediate use.

4.22 Primary secondary amine (PSA) filler: Particle size of 40 μ m~60 μ m.

5 Instruments

5.1

High performance liquid chromatograph

Equipped with ultraviolet detector.

5.2

Analytical balance

Sensitivity of 0.01g.

5.3

Analytical balance

Sensitivity of 0.0001g.

5.4

Analytical balance

Sensitivity of 0.00001g.

5.5

Ultrasonic cleaner.

5.6

Centrifuge

Rotating speed of 8000 r/min.

5.7

Vortex mixer.

6.1 Preparation of specimen

Take a representative specimen and implement it in accordance with the provisions of GB/T 30891.

6.2.1 Fish and crustaceans

Take 2g (accurate to 0.01g) of the specimen into a 50mL centrifuge tube; add 4g of anhydrous MgSO_4 ; then add 10mL of acetone; vortex fully. Perform the ultrasonic

Where:

X - astaxanthin content in the sample, in mg/kg;

1.3 - correction factor of 13-cis astaxanthin against all-trans astaxanthin;

A_{13-cis} - peak area of 13-cis astaxanthin in the specimen solution;

A_{trans} - peak area of all-trans astaxanthin in the specimen solution;

1.1 - correction factor of 9-cis astaxanthin against all-trans astaxanthin;

A_{9-cis} - peak area of 9-cis astaxanthin in the specimen solution;

C_s - all-trans astaxanthin content in the standard working solution, in $\mu\text{g/mL}$;

V - volume of specimen solution, in mL;

A_s - peak area of all-trans astaxanthin standard working solution;

m - mass of sample, in g;

f - dilution rate.

8 Method Quantification Limit, Recovery Rate and

Precision

8.1 Quantification limit

The quantification limit of astaxanthin in fish and crustaceans is 2.5mg/kg; the quantification limit of astaxanthin in shrimp meal is 5mg/kg; and the quantification limit of astaxanthin in

krill oil is 10mg/kg.

8.2 Recovery rate

When the addition concentration of this method is 2.5mg/kg~100mg/kg, the recovery rate shall be 90%~110%.

8.3 Precision

The inter-assay coefficient of variation of this method is $\leq 10\%$; and the intra-assay coefficient of variation is $\leq 10\%$.

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