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Determination of L-ascorbyl-2-monophosphate in feeds - High performance liquid chromatography

饲料中 L-抗坏血酸-2-磷酸酯的测定 高效液相色谱法

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1 Scope

This Standard specifies the high performance liquid chromatography detection method for L-ascorbyl-2-monophosphate in feeds.

This Standard applies to the determination of L-ascorbyl-2-monophosphate content in compound feeds, concentrated feeds and additive premixed feed for aquatic products. The detection-limit of the method is 2 mg/kg (calculated as L- ascorbic acid), and the quantitation-limit is 10 mg/kg (calculated as L- ascorbic acid).

2 Normative references

The terms in the following documents become the terms of this Standard by reference to this Standard. For dated references, all subsequent amendments (not including errata content) or revisions do not apply to this standard. However, parties to agreements that are based on this Standard are encouraged to study whether the latest versions of these documents can be used. For undated references, the latest edition applies to this Standard.

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

GB/T 14699.1, Feeding stuffs - Sampling

GB/T 20195, Animal feeding stuffs - Preparation of test samples

3 Principle

The L-ascorbyl-2-monophosphate in the sample shall be extracted with phosphate buffer solution, centrifuged and filtered; the filtrate shall be measured with high performance liquid chromatography, and quantified by external standard method.

4 Reagents or materials

Unless otherwise stated, in the analysis, only use reagents confirmed to be analytical reagents AND water complying with the grade-II water specified in GB/T 6682.

4.1 Methanol: chromatographic pure.

7 Sample preparation

Prepare the sample according to GB/T 20195; grind it; pass it through a 0.45 mm mesh sieve; mix it evenly; put it in an airtight container; store it at low temperature in the dark for future use.

8 Analysis steps

8.1 Extraction

Weigh a certain amount of sample [containing about 1 mg of L-ascorbyl-2-monophosphate (calculated as L- ascorbic acid)], accurate to 0.1 mg; put it in a 100 mL volumetric flask; add about 70 mL of phosphate buffered saline (4.5); sonicate in an ultrasonic water bath for 15 minutes; use phosphate buffered saline (4.5) to dilute to the mark (V). Take an appropriate amount of solution and centrifuge at 3 500 r/min for 5 min. After centrifugation, filter the supernatant through a 0.45 μm filter membrane; use the filtrate for high-performance liquid chromatography analysis.

8.2 Determination

8.2.1 Liquid chromatography reference conditions

Chromatographic column: C_{18} column, length 250 mm, inner diameter 4.6 mm, particle size 5 μm, or equivalent performance.

Column temperature: room temperature.

Flow rate: 1.0 mL/min.

Detection wavelength: 250 nm.

Injection volume: 20 μL.

8.2.2 Drawing of the standard curve

Continuously inject L-ascorbyl-2-monophosphate standard working solution (4.8) into the HPLC analyzer with a stable baseline, from low concentration to high; plot the peak area-concentration to obtain the standard curve regression equation.

8.2.3 Quantitative determination

Inject the sample solution to be tested (8.1), in which the peak area response value of L-ascorbyl-2-monophosphate shall be within the linear range of the standard curve; if it exceeds the linear range, it shall be diluted and then injected for analysis. According to the peak area, obtain the concentration (C) of L-ascorbyl-2-monophosphate in the

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