



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

GB 1886.99-2015

**National Food Safety Standard -- Food Additives --
L-?-aspartyl-N-(2,2,4,4-tetramethyl-3-trimethylene sulfide)
-D-alaninamide (also known as alitame)**

食品安全国家标准 食品添加剂 L- α -天冬氨酰-N- (2,2,4,4-四甲基-3-硫化三亚甲基)
-D-丙氨酰胺 (又名阿力甜)

(English Translation)

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1 Scope

This standard applies to food additives L-alpha- aspartyl -N- (2,2,4,4- tetramethyl-3 trimethylene sulfide) -D- alanine amide (aka Alitame).

2.1 Chemical Name

L-alpha- aspartyl -N- (2,2,4,4- tetramethyl-3 trimethylene sulfide) -D- alaninamide Formula
2.2 $C_{14}H_{25}N_3O_4S \cdot 2.5H_2O$

2.4 Relative molecular mass

376.47 (according to 2007 international relative atomic mass)

3.1 Sensory requirements

Sensory requirements shall comply with the requirements of Table 1.

Table 1 Sensory requirements

Project requires test methods Color White Status crystalline powder Odorless or slight odor distinctive odor.

The sample was placed in a clean, dry white porcelain dish, under natural light, observation the state of its color, smell its odor

3.2 Physical and Chemical Indicators

Physical and chemical indicators should be consistent with the provisions of Table 2.

Table 2. Physical and chemical indicators

Item Index Test Method Alitame content (dry basis), w /% 98.0 ~ 101.0 Appendix A A.3 beta- isomer (dry basis), w /% ≤ 0.3 Appendix A A.3 Alanine amide (dry basis), w /% ≤ 0.2 Appendix A A.3 Water, w /% 11 ~ 13 GB/T 6283 Specific rotation α_D^{25} [(°) · dm² · kg⁻¹] 40 ~ 50 in Appendix A A.4 Residue on ignition, w /% ≤ 1.0 GB/T 9741a Lead (Pb)/(mg/kg) ≤ 1.0 GB 5009.12 a burning temperature of 800 °C ± 25 °C.

Appendix A

Testing method

A.1 General Provisions this standard reagents and water in the absence of other specified requirements, refer to the three water analytical reagent and GB/T 6682 regulations. test in the standard solution, standard solution for measuring impurities, formulations and products, did not indicate when the other requirements according to GB/T 601, GB/T 602 with and the provisions of GB/T 603 was prepared. Solution was used in the tests did not indicate what is formulated with solvent, it refers to an aqueous solution.

A.2 Identification Test A.2.1 solubility Soluble in water and ethanol.

A.2.2 IR spectra

IR spectra consistent with Figure B.1.

A.2.3 pH

pH 50g/L of sample solution is 5.0 to 6.0.

A.2.4 color reaction A.2.4.1 Weigh 300mg ninhydrin added 100mL of n-butanol and 2mL glacial acetic acid formulated as a solution, of this solution 5mL, added 10mg sample was slowly heated to reflux, should appear bright blue-purple.

A.2.4.2 Take 5mL freshly prepared c (5KMnO₄) = Potassium permanganate standard solution 0.001mol/L, add 10mg sample and filled Well mixed, the solution from purple to brown.

Determination A.3 alitame (dry basis) content, beta- isomers and alanine amide A.3.1 Method summary Ion-pair reversed-phase HPLC method under the selected operating conditions, the sample solution through the column in each fractionation, UV absorption detector, using external standard, calculate the content of the samples alitame, beta-isomers and alanine amide.

A.3.2 Reagents and materials A.3.2.1 methanol.

A.3.2.2 Acetonitrile. HPLC grade, at 210nm the light transmittance is greater than 90%.

A.3.2.3 beta- isomer. standard sample.

A.3.2.4 alanine amide. standard sample.

A.3.2.5 alitame. standard sample.

A.3.2.6 buffer solution. Weigh 0.69g sodium phosphate monobasic (monohydrate) and 4.32g1- octyl sulfate, accurate to 0.0001g, placed 1000mL volumetric flask. 200mL of water was added to dissolve adjusted to pH 2.5 with phosphoric acid, diluted with water to the mark. By pore size 0.22 μ m millipore membrane filter or equivalent.

A.3.3 Instruments and Equipment A.3.3.1 high performance liquid chromatography system (HPLC).

A.3.3.2 constant flow pump.

A.3.3.3 UV absorbance detector.

A.3.4 Chromatography conditions Recommended columns and typical operating conditions are shown in Table A.1.

Table A.1 column chromatography and typical operating conditions

C18 column 15cm x 0.4cm Column temperature room temperature Appendix A mobile phase in A.3.5.1 Flow rate/(mL/min) 1.0 Detector wavelength/nm 217 A.3.5 Analysis step A.3.5.1 Preparation of the mobile phase Accurately taken acetonitrile and buffer solution volume ratio 1.3 mixed well. By vacuum degassing.

A.3.5.2 Preparation of standard working solution A.3.5.2.1 standard solution A1. Weigh beta- alanine amide isomers and each about 25mg, accurate to 0.0001g, moved 500mL capacity Bottle. 50mL methanol was added to dissolve, dilute to the mark with water. Stored in a refrigerator.

A.3.5.2.2 standard solution A2. Pipette 15mL $\pm$ 0.02mL standard solution A1 to 50mL volumetric flask, diluted with water to the mark.

A.3.5.2.3 standard solution W1. Weigh about 50mg alitame standard samples, accurate to 0.0001g, moved into 10mL volumetric flask, Add 5mL standard solution A2, diluted with water to the mark.

A.3.5.2.4 standard solution W2. pipette 5mL $\pm$ 0.02mL standard solution W1 to 50mL volumetric flask, dilute with water to Scale.

A.3.5.3 Preparation of test solution A.3.5.3.1 Test solution S1. Weigh about 50mg samples, accurate to 0.0001g, set 10mL volumetric flask, dilute to the mark.

A.3.5.3.2 test solution S2. pipette 5mL $\pm$ 0.02mL test solution S1 to 50mL volumetric flask, diluted with water to the mark.

System suitability test A.3.5.4 by HPLC procedures warm-up, adjust the temperature and flow, to analyze the conditions and steady baseline. Repeat 3 times, respectively, injection 100 μ L into standard solution and standard solution W1 W2, beta- isomer, alitame and alanine amides such as approximate retention time

Table A.2. The coefficient of variation of the peak area (100 times standard deviation

divided by the average peak area) should not exceed 2%.

Table A.2 approximate retention times of the components

Ingredient name Approximate retention times/min beta- isomer 6 Alitame 10 Alanine amide 15
 Note. The retention time of approximately 15cm x 0.4cm column obtained. If you use other columns or different lengths should be adjusted in the mobile phase of acetonitrile Ratio to obtain the retention time required.

A.3.5.5 Determination the mobile phase was pumped into the column to baseline no longer drift. Standard solution and the test solution was analyzed under the system suitability test conditions.

Injection three times the standard working solution W1, beta- isomers and calculate the average peak area alanine amide. Injection three times standard solution W2, calculate the average peak area of alitame. Inject test solution three times S1, beta- isomers and calculate the average peak area alanine amide.

Three times injection test solution S2, calculate the average peak area of alitame.

A.3.6 Calculation Results Alitame content (dry basis) of the mass fraction w1, according to equation (A.1) Calculated.

$RA \times WS \times PS$

$RS \times WA \times$

100% (A.1)

Where.

RA --- test solution S2 detected peak response value;

WS --- alitame standard samples after subtracting the mass of water, in grams (g);

PS --- beta- alanine amide isomer or purity standard sample,%;

Response value RS --- standard working solution W2 detected peak;

WA --- sample after subtracting the mass of water, in grams (g).

beta- isomer (dry basis) of the mass fraction w2, according to equation (A.2) Calculated.

$RA \times WS \times PS \times DF$

$RS \times WA \times$

100% (A.2)

Where.

RA --- test solution S1 detected peak response value;