ALITAME

Prepared at the 59th JECFA (2002), published in FNP 52 Add 10 (2002) superseding specifications prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996), and incorporating heavy metal limits established by the 57th JECFA (2001), published in FNP 52 Add 9 (2001). An ADI of 0-1 mg/kg bw was established at the 46th JECFA (1996).

SYNONYMS INS No. 956

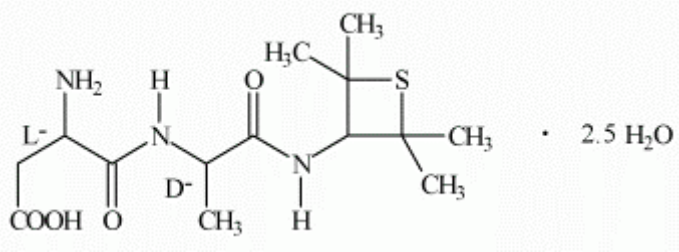
DEFINITION Alitame is prepared by a multistep synthesis involving the reaction between two intermediates, (S)-[2,5-dioxo-(4-thiazolidine)] acetic acid and (R)-2-amino-N-(2,2,4,4-tetramethyl-3-thietanyl)propanamide. The final product is isolated and purified through crystallization of an alitame / 4-methylbenzenesulfonic acid adduct followed by additional purification steps, and finally recrystallization from water as the 2.5 hydrate.

Chemical names L-α-Aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide, hydrated

C.A.S. number 99016-42-9 (hydrated form)
80863-62-3 (anhydrous form)

Chemical formula $C_{14}H_{25}N_3O_4S \cdot 2.5\ H_2O$

Structural formula



Formula weight 376.5 (hydrated form)

Assay Not less than 98.0% and not more than 101.0% on the anhydrous basis

DESCRIPTION White, crystalline powder, odourless or having a slight characteristic odour. Approximately 2000 times sweeter than sucrose.

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water and in ethanol

Infrared spectrum (Vol. 4) The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum below

pH (Vol. 4) Between 5.0 and 6.0 (5 in 100 soln)

Colour reactions

1. To 5 ml of a solution of 300 mg of ninhydrin in 100 ml of n-butanol and 2 ml of glacial acetic acid, add 10 mg of the sample, and heat to gentle reflux. An intense blue-violet colour is formed.

2. To 5 ml of a freshly prepared 0.001 mol/l potassium permanganate solution add 10 mg of the sample and mix thoroughly. The purple solution changes to brown.

PURITY

Beta isomer

Not more than 0.3%, calculated on the anhydrous basis
See description under METHOD OF ASSAY

Alanine amide

Not more than 0.2%, calculated on the anhydrous basis
See description under METHOD OF ASSAY

Water (Vol. 4)

Between 11 and 13% (Karl Fischer method)

Specific rotation (Vol. 4)

[α] 25, D: between +40º and +50º, 1% (w/v) in water

Sulfated ash (Vol. 4)

Not more than 1.0%

Lead

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Principle

Alitame and its constituents, the beta isomer and alanine amide, are measured by reverse-phase ion-pair HPLC.

Chromatographic System

Fit a high pressure liquid chromatograph, operated at room temperature, with a constant flow pump and a 15 x 0.4 cm NovaPak C_{18} reverse phase ion-pair column (Waters, or equivalent). The mobile phase is maintained at a pressure and flow rate (typically 1.0 ml/min) capable of giving the required elution time (see System Suitability Test). An ultraviolet detector that monitors absorption at 217 nm is used.

Mobile Phase

To make the buffer solution, add 0.69 g of sodium phosphate, monobasic, monohydrate and 4.32 g sodium 1-octanesulfonate, reagent grade (obtainable from Regis Chemical Co.) to a 1000-ml volumetric flask. Add 200 ml of water, stir to dissolve the salts and adjust the pH to 2.5 with phosphoric acid (85%, reagent grade). Add water to volume. Filter through 0.22 µm Millipore filter or equivalent. Accurately measure one part by volume of acetonitrile (LC grade, transmittance more than 90% at 210 nm) and three parts by volume of buffer solution, and combine. De-gas under vacuum.

Standard Solution A1

Weigh accurately about 25 mg each of the beta isomer and alanine amide

(both obtainable from Quality Control Department, Danisco Sweeteners, PO Box 8266, Terre Haute, Indiana 47808, USA), and transfer quantitatively to a 500 ml volumetric flask. Add 50 ml methanol to aid dissolution, and dilute with water to volume. Store in a refrigerator.

Standard Solution A2
Transfer 15.0 ml of Standard Solution A1 into a 50 ml volumetric flask and dilute with water to volume.

Working Standard W1
Weigh accurately about 50 mg Alitame Reference Standard (obtainable from Quality Control Department, Danisco Sweeteners, PO Box 8266, Terre Haute, Indiana 47808, USA), transfer quantitatively to a 10 ml volumetric flask, add 5 ml of Standard Solution A2, and dilute with water to volume.

Working Standard W2
Transfer 5.0 ml of Working Standard W1 to a 50 ml volumetric flask and dilute with water to volume.

Test Solution S1
Weigh accurately about 50 mg of the sample, transfer quantitatively to a 10 ml volumetric flask and dilute with water to volume.

Test Solution S2
Transfer 5.0 ml of Test Solution S1 to a 50 ml volumetric flask and dilute with water to volume.

System Suitability Test
Inject triplicate 100 µl portions of Working Standards W1 and W2 into the chromatograph. The retention times for the beta isomer, alitame, and alanine amide should be approximately 6, 10, and 15 min respectively. (Note: The retention time quoted is appropriate for a 15 x 0.4 cm NovaPak column. If a column of a different make or length is used, it may be necessary to adjust the proportion of acetonitrile in the eluent to obtain the required retention time). The coefficient of variation (100 x standard deviation divided by mean peak area) for the peak areas should not exceed 2%.

Procedure
Equilibrate the column by pumping mobile phase through it until a drift-free baseline is obtained. Analyze the Standard Solutions and Test Solutions under the conditions described above. Inject three replicate samples of Working Standard W1, and calculate the average peak areas for the beta isomer and for alanine amide. Inject three replicate samples of Working Standard W2 and calculate the average peak area for alitame. Inject three replicate samples of Test Solution S1 and calculate the average peak areas for the beta isomer and alanine amide. Inject three replicate samples of Test Solution S2 and calculate the average peak area for alitame.
Calculate the purity of alitame by the formula:

$$Wt\,\% = \frac{R_A \, x \, W_S \, x \, P_S}{R_S \, x \, W_A}$$

where
R_A = the response of the analyte peak in Test Solution S2

W_S = the weight of the alitame Reference Standard corrected for water content, in g
P_S = the percent purity of the Reference Standard, i.e., 100.00 - sum of impurities
R_S = the response of the analyte peak in Working Standard W2
W_A = the weight of the sample corrected for the water content, in g.
Calculate the percentage of the beta isomer and alanine amide by the following formula:

$$Wt\,\% = \frac{R_A \times W_S \times P_S \times DF}{R_S \times W_A}$$

where
R_A = the response of the analyte peak in Test Solution S1
W_S = the weight of the beta isomer or alanine amide standards, uncorrected for water content, in g
P_S = the percentage purity of the beta isomer or alanine amide standard, i.e. 100.00 - sum of impurities
R_S = the response of the analyte peak in Working Standard W1
W_A = the weight of the sample, uncorrected for water content, in g
DF = the dilution factor, i.e. 0.003.

Infrared spectra of Alitame