# ASPARTAME-ACESULFAME SALT

*Revised specifications prepared at the 65<sup>th</sup> JECFA (2005) and published in FNP 52 Add 13 (2005), superseding specifications prepared at the 55<sup>th</sup> JECFA (2000) and published in FNP 52 Add 8 (2000). The ADIs for aspartame (0-40 mg/kg bw) established at the 25<sup>th</sup> JECFA (1981) and for acesulfame K (0-15 mg/kg bw) established at the 37<sup>th</sup> JECFA (1990) cover the aspartame and acesulfame moieties of the salt.*

**SYNONYMS**          Aspartame-acesulfame, INS No. 962

**DEFINITION**        The salt is prepared by heating an approximately 2:1 ratio (w:w) of aspartame and acesulfame K in solution at acidic pH and allowing crystallization to occur. The potassium and moisture are eliminated. The product is more stable than aspartame alone.

Chemical names        6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L-$\alpha$-aspartic acid.
[2-carboxy-β-(*N*-(*b*-methoxycarbonyl-2-phenyl)ethylcarbamoyl)]ethanaminium-6-methyl-4-oxo-1,2,3-oxathiazin-3-ide-2,2-dioxide.
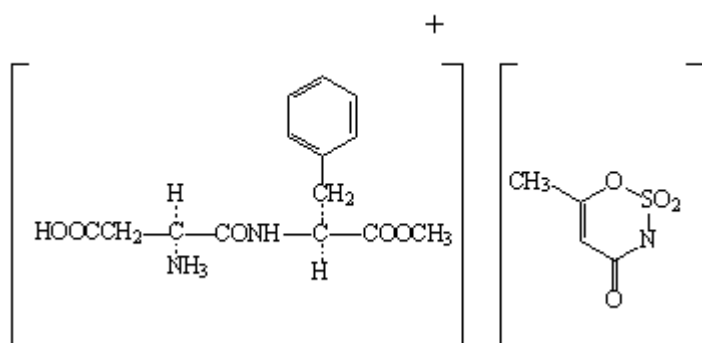
C.A.S. number         106372-55-8

Chemical formula      $C_{18}H_{23}O_9N_3S$

Structural formula



Formula weight        457.46

Assay                 63.0% to 66.0% aspartame (dried basis) and 34.0% to 37.0% acesulfame (acid form on a dried basis).

**DESCRIPTION**       A white, odourless, crystalline powder

**FUNCTIONAL USES**   Sweetening agent, flavour enhancer

## CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4))          Sparingly soluble in water, and slightly soluble in ethanol.

PURITY

| | |
|---|---|
| <u>Loss on drying</u> (Vol. 4) | No more than 0.5% (105°, 4 h) |
| <u>Transmittance</u> (Vol. 4) | The transmittance of a 1% solution in water determined in a 1 cm cell at 430 nm with a suitable spectrophotometer using water as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022. |
| <u>Specific Rotation</u> (Vol. 4) | $[\alpha]_D^{20}$ +14.5 to +16.5.<br>After preparing a solution of 6.2 g of sample in 100 ml formic acid (15N), make the measurement within 30 min of preparation of the solution. Divide the calculated specific rotation by 0.646 to correct for the aspartame content of the aspartame-acesulfame salt. |
| <u>5-Benzyl-3,6-dioxo-2-piperazineacetic acid</u> | Not more than 0.5%<br>See description under TESTS |
| <u>Lead</u> (Vol. 4) | Not more than 1 mg/kg.<br>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." |

## TESTS

<u>5-Benzyl-3,6-dioxo- 2-piperazine acetic acid</u>

<u>Principle</u>
5-benzyl-3,6-dioxo- 2-piperazine acetic acid is determined in aspartame-acesulfame salt dissolved in methanol-water by comparison to an authentic standard after separation by HPLC.

<u>Apparatus</u>
Use a suitable high-pressure liquid chromatograph equipped with UV detector for measuring absorbance at 210 nm and a 250 x 4.6 mm column packed with octyldecyl silanized silica (10-$\mu$m Partisil ODS-3 or equivalent) and operated under isocratic conditions at 40°.

<u>Mobile phase</u>
Dissolve 5.6 g of potassium phosphate monobasic into 820 ml of water in a 1-l flask and adjust the pH to 4.3 with phosphoric acid. Add 180 ml of methanol and mix. Filter through a 0.45 $\mu$m filter and de-gas.

<u>Standard</u>
Accurately weigh approximately 25 mg of authentic 5-benzyl-3,6-dioxo-2-piperazine acetic acid into a 100-ml volumetric flask; add 10 ml of methanol to dissolve the material and dilute to volume with water and mix. Accurately transfer 15 ml of this solution to a 50-ml flask and dilute to volume with a 1:9 (v:v) mixture of methanol:water prepared on the day of use.

<u>Sample</u>
Accurately weigh approximately 50 mg of sample into a 10 ml volumetric flask and dilute to volume with a 1:9 (v:v) mixture of methanol:water prepared on the day of use.

<u>Procedure</u>

Separately inject 20 μl portions of the standard and the sample into the chromatograph (The flow rate of the mobile phase is about 2 ml/min.) and record the peak areas in standard and sample chromatograms (under the conditions described, the retention time of 5-benzyl-3,6-dioxo-2-piperazine acetic acid and aspartame are approximately 4 and 11 min, respectively). Measure the peak area response of 5-benzyl-3,6-dioxo- 2-piperazine acetic acid in each chromatogram and calculate the percentage of 5-benzyl-3,6-dioxo-2-piperazine acetic acid as follows:

$$\% = 1000(A_U C_S)/(A_S W_u)$$

Where $A_U$ and $A_S$ are the peak areas of 5-benzyl-3,6-dioxo-2-piperazine acetic acid in the sample and standard, respectively, $C_S$ is the concentration of 5-benzyl-3,6-dioxo-2-piperazine acetic acid in the standard in mg/ml and $W_u$ is the weight, in mg, of aspartame-acesulfame salt taken in the sample preparation.

**METHOD OF ASSAY**

Principle
Aspartame-acesulfame salt is dissolved in methanol and potentiometrically titrated with tetrabutylammonium hydroxide.

Apparatus
Use a suitable autotitrator (e.g., Metrohm 670, or equivalent) equipped with a glass pH electrode and a silver-silver chloride double liquid junction reference electrode (e.g., Yokogawa pH electrode SM 21-AL4 or equivalent and reference electrode SR 20-AS52 or equivalent).

Standard tetrabutylammonium hydroxide solution
Prepare a 0.1 M solution in a 1:1 (v:v) mixture of 2-propanol:methanol. Weigh 24 and 98 mg benzoic acid with 0.01 mg accuracy and dissolve each into two 50-ml volumetric flasks and dilute to volume with 2-propanol. Titrate both solutions with the 0.1 M tetrabutylammonium hydroxide and record the volume required to reach the equivalence point with 0.001 ml accuracy. Perform a blank titration on 50 ml of 2-propanol. Determine the standard factor (F) for each titration. and average the two factors as follows:

$$F = [(W_S \times 1000)/(122 \times (V_S - V_O))]$$

Where:  $W_S$ = weight of primary benzoic acid (g)
  $V_S$ = volume of equivalence point (ml)
  $V_o$ = volume of equivalence for the blank (ml)
  122 = molecular weight of benzoic acid

Procedure
Weigh accurately 100 to 150 mg of sample and dissolve it in 50-ml methanol. Titrate with the standardized 0.1 M tetrabutylammonium hydroxide. Determine the volume (ml) of the standard solution needed to reach the first (V1) and second (V2) equivalency points. Perform a blank titration on the methanol. Calculate the acesulfame and aspartame content as follows:

Acesulfame content (% m/m) = $[(V_1 - V_B) \times N \times 163/(10 \times W)]$

Aspartame content (% m/m) = $[(V_2 - V_1) \times N \times 294/(10 \times W)]$

Where:  W = Weight of sample (g)

$V_1$ = volume of first equivalence point (ml)

$V_2$ = volume of second equivalence point (ml)

$V_B$ = volume of equivalence point of blank (m)l

N = normality of the standard 0.1 M tetrabutylammonium hydroxide

163 and 294 = formula weights of acesulfame and aspartame moieties, respectively

10 = conversion of g to % (m/m)