Test method for aspartic acid

Related substances(Dicarboxylic acid)

Diluent: Take 100ml of 6mol/L hydrochloric acid and dilute with water to 1000ml.。

Test solution: Take an appropriate amount of this product, weigh it accurately, dissolve it in diluent and dilute it to make a solution containing about 50mg per 1ml.

Reference solution: Take appropriate amounts of malic acid reference substance and maleic acid reference substance, accurately weigh them, dissolve and dilute them with diluent to prepare a mixed solution containing approximately 100 μg/ml of malic acid and 50 μg/ml of maleic acid .

Fumaric acid reference solution: Take an appropriate amount of fumaric acid reference substance, weigh accurately, dissolve and dilute with diluent to prepare a solution containing 50 μg/ml .

System suitability solution: Take appropriate amounts of malic acid reference substance, maleic acid reference substance, and fumaric acid reference substance, accurately weigh them, dissolve and dilute them with a diluent to prepare a mixed solution containing approximately 100 μg/ml of malic acid, 50 μg/mlof maleic acid, and 50 μg/ml of fumaric acid.

Chromatographic conditions: Use a strong cation exchange column; Using 0.39g/L sulfuric acid aqueous solution as the mobile phase, the detection wavelength is 210nm; the column temperature is 30℃, the flow rate is 0.6ml per minute, and the injection volume is 10μl.

System Applicability Requirements: In the system suitability solution chromatogram, the peak sequence is maleic acid, malic acid, and fumaric acid, and the separation degree between each peak should comply with the regulations. The theoretical number of plates for the maleic acid peak should not be less than 3000.

Determination method: Precisely measure the reference solution and the test solution, inject them into the liquid chromatograph separately, and record the chromatogram.

Limit: Calculate the content of each known impurity based on peak area using the external standard method. The content of malic acid should not exceed 0.2%, maleic acid should not exceed 0.1%, and fumaric acid should not exceed 0.1%; Single unknown impurity shall be calculated based on the peak area of malic acid using the external standard method, and shall not exceed 0.1%. The total amount of impurities shall not exceed 0.5%.

Other amino acids

Sample Diluent: Take 300ml of water, add 0.7ml of hydrochloric acid, and dilute with water to 1000ml.

Test solution: Take an appropriate amount of the test sample, weigh it accurately, dissolve and dilute it with sample diluent to prepare a solution containing approximately 1.5mg of aspartic acid per 1ml.

Reference solution: Accurately measure 1ml of the test solution, place it in a 100ml volumetric flask, dilute to the mark with sample diluent, and shake well. Accurately measure 1ml and place it in a 10ml volumetric flask. Dilute to the mark with sample diluent and shake well.

Proline reference solution: Take an appropriate amount of proline reference solution, weigh it accurately, dissolve and dilute it with sample diluent to prepare a solution containing 1.5μg/ml of proline .

System suitability solution: Take appropriate amounts of isoleucine and leucine reference standards, weigh them accurately, dissolve and dilute them with sample diluent to prepare a mixed solution containing 3μg/ml of isoleucine and 3μg/ml of leucine.

Reference solution: Take appropriate amounts of alanine reference substance, asparagine reference substance, and glutamic acid reference substance, accurately weigh them, dissolve and dilute them with sample diluent to prepare a mixed solution containing approximately 0.75μg/ml of alanine, 0.75 μg/ml of asparagine, and 0.75 μg/ml of glutamic acid.

Use a suitable amino acid analyzer for measurement.

System Applicability Requirements: The separation degree of isoleucine and leucine peaks in the system suitability solution chromatogram should not be less than 1.2.

Determination method: Accurately measure the applicability solution of the system, proline reference solution, reference solution, reference solution, and test solution, and inject them into the amino acid analyzer respectively. Record the chromatogram.

Limit: Calculate the content of each known impurity based on peak area using the external standard method. The content of glutamic acid should not exceed 0.05%, alanine should not exceed 0.05%, and asparagine should not exceed 0.05%; Other nintrine positive substances detected at 570nm are calculated based on the peak area of aspartic acid in the control solution, while other nintrine positive substances detected at 440nm are calculated based on the peak area of proline in the proline solution (if the impurity peak values detected at 570nm and 440nm are both greater than 0.05%, the results are reported at 570nm); Single unknown impurity shall not exceed 0.10%, and the total amount of impurities shall not exceed 1.0%.