



Designation: E 1759 – 95

Standard Test Method for Isoaspartic Acid in Proteins: Method for the Determination of Asparagine Deamidation Products¹

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INTRODUCTION

The storage of proteins in aqueous solutions often results in the formation of isoaspartic acid linkages within the polypeptide chain as a result of the deamidation of asparagine residues and the rearrangement of aspartic acid linkages. This test measures the amount of isoaspartic acid residues in a protein or peptide solution by the use of the enzyme protein isoaspartyl methyl transferase and radioactive S-adenosyl-L-methionine.

1. Scope

1.1 This test method covers the determination of isoaspartic acid residues in a protein or peptide sample. This test method is applicable for the determination of isoaspartic acid residues in a sample in the range of 2.5–50 μmol/L. Higher concentrations can be determined following dilution. The reported lower range is based on single-operator precision.

1.2 The values stated in SI units are to be regarded as the standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Terminology

2.1 Definitions of Terms Specific to This Standard:

2.1.1 *isoaspartic acid residue*—indicates an aspartic acid residue in which linkage of the polypeptide chain takes place through the gamma carboxyl group of the aspartic acid versus the alpha carboxyl group that is used in the normal peptide linkage.

3. Summary of Test Method

3.1 The basis of the procedure given in this test method is the production of radioactive methanol equal to the amount of isoaspartic acid residues present in a protein sample through the action of the enzyme protein isoaspartyl methyl transferase and radiolabelled S-adenosyl-L-methionine, a radiolabelled

form of a co-factor that is consumed in the enzymatic reaction of the enzyme. During the test a radiolabelled intermediate is formed through the transfer of the labeled methyl group from S-adenosyl-L-methionine to the alpha carboxy group of isoaspartic acid. This methylated intermediate is then degraded to liberate the methyl group as methanol. The methanol is then captured in a methanol diffusion procedure and counted.

3.2 A sample of protein is incubated with the enzyme protein isoaspartyl methyl transferase and radiolabelled S-Adenosyl Methionine in a buffer that results in the accumulation of the methyl esters of isoaspartic acid residues through the enzymatic transfer of the methyl group from S-adenosyl-L-methionine to isoaspartic acid sites in the protein. The protein solution is then treated with a basic solution containing sodium dodecyl sulfate in order to inactivate the enzyme and convert the methylated isoaspartic acid residues to a succinimide and free methanol. The methanol is then separated from the protein solution through the diffusion of the methanol to a scintillation fluid solution. The methanol transferred to the scintillation fluid is then determined by counting of the radioactivity in the scintillation fluid.

4. Significance and Use

4.1 Isoaspartic acid residues are generated during incubation of proteins under a wide variety of conditions in aqueous solution. Such residues are generated most commonly through the deamidation of asparagine residues although some reports of isoaspartic acid formation through the rearrangement of aspartic acid residues have been published.

4.2 The presence of such residues can indicate that the protein containing such residues has suffered damage that may affect the biological activity of the protein. The precise

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