

Hydrolysis method

Samples are dried in pyrolyzed borosilicate hydrolysis tubes in a vacuum centrifugal concentrator and subjected to gas phase hydrolysis by 6N HCl with 1% phenol at 110°C for 20-24 hours under vacuum. The internal standard norleucine is added to samples prior to hydrolysis. After hydrolysis, excess HCl is removed by vacuum and amino acids are redissolved in citrate buffer and transferred to injection vials which are loaded into the autosampler for automatic injection.

Limitations

- Acid hydrolysis converts asparagine and glutamine to aspartic and glutamic acid respectively. That is, the amino acid analysis result for Asp is a total of Asp + Asn and the result for Glu is Glu + Gln.
- Hydroxyl group in threonine and serine promote the breakdown of the residue: losses are 5-10% and 10-15%, respectively, after 24 hours.
- Methionine may be oxidized to methionine sulfoxide and methionine sulfone.
- Tyrosine can undergo halogenation during HCl hydrolysis. If iron or copper ions are present, tyrosine recoveries can be low. High fat content (>5%) can also lead to reduced yields.
- Tryptophan and cysteine are destroyed under acid hydrolysis. Cys can be determined by oxidation to cysteic acid or by reduction and alkylation prior to the standard acid hydrolysis. Two alternate hydrolysis strategies for quantifying tryptophan are hydrolysis in the presence of dodecanethiol and hydrolysis with 4 N methanesulfonic acid.
- Groups of aliphatic amino acids such as Val-Val, Ile-Ile, Val-Ile or Ala-Ala are exceptionally difficult to cleave with acid, and often require longer hydrolysis time.
- Phosphoamino acids loss the phosphate acid labile moiety during hydrolysis.
- Glycosylated amino acids convert to complex by-products of very difficult identification.