Waters
Lab Highlights
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Loss of Amino Acids During Protein Hydrolysis

The PICO-TAG™ Amino Acid Analysis System provides state-of-the-art performance for protein compositional analysis. This performance can be compromised, however, if inadequate attention is paid to hydrolysis procedures. Most researchers in the field of amino acid analysis are aware that tryptophan is largely destroyed during acid hydrolysis of proteins, and that cystine is partly converted to cysteic acid[1]. It is also well known that serine, threonine, and tyrosine are partially destroyed; careful workers often make corrections for these losses. Fewer people recognize that other amino acids besides those mentioned above can suffer degradation to a greater or lesser degree, and that hydrolysis in the presence of non-amino acid species may have unforeseen consequences.

In the simplest of cases, where a pure protein, salt-free and without prosthetic or conjugated groups, is subjected to hydrolysis, the only significant interaction is between the reagent and each amino acid. The presence of other species, however, including: anions (sulfate, phosphate); cations (sodium, potassium, calcium); heavy metals (copper, iron); organic groups (sugars, porphyrins & other heterocycles, lipids) may lead to altered rates of hydrolysis, formation of artifacts, and loss of specific amino acids.

Time required for hydrolysis varies from sample to sample. Some proteins require extended times for complete hydrolysis; sequences such as Leu-Leu, Ile-Leu, Ile-Val, and Val-Gly are extremely resistant to cleavage. In contrast, Asp is released very rapidly; this may result in its spending far more time in solution than other amino acids, and thus being more vulnerable to secondary reactions.

Following are several precautions which should be observed to assure that you get the most out of your high performance PICO-TAG™ System.

- Use only high quality reagent (Pierce Constant Boiling Hydrochloric Acid). Lower grade HCl may be contaminated with amino acids, ammonia, iron, copper, bromine and other trace materials. Use only Milli-Q® (or equivalent) water for dilution of samples.
- Keep the reagent well sealed, and do not use for any other purpose.
- Use only high quality borosilicate glass tubes (Waters P/N 07571). Use of low quality glass, or glass which has been scratched or etched will lead to poor results.
- Remove excess HCl from samples *immediately* following hydrolysis.

1. S. Hunt (1985) in *Chemistry and Biochemistry of the Amino Acids*, (ed. G. C. Barrett) Chapman and Hall, London, pp 377-398.

Tom Tarvin

MILLIPORE
Waters Chromatography Division 34 Maple St. / Milford, MA 01757 / 617-478-2000