PRODUCT INFORMATION AMINO ACID ANALYSIS

High-sensitivity amino acid analysis of protein hydrolysates is performed using an automated two-step pre-column derivatization with two different reagents: OPA (o-phthalaldehyde) for primary, and FMOC (9-fluorenylmethylchloroformate) for secondary amino acids. Derivatization is followed by separation, detection and quantification of the derivatives, using dedicated HPLC equipment (Aminoquant method [1]). In case of D,L separation [2], a pre-column OPA derivatization in combination with a chiral thiol compound (IBLC; iso-butyryl-L-cysteine) is used to obtain stereo selectivity; consequently Pro is not quantified.

Our standard hydrolysis conditions, 5.7 N HCl in the gas-phase for 1.5 hours at 150^{o}C, results in a good balance between destruction of oxidation-sensitive amino acids and recovery of hydrophobic amino acids. Acid hydrolysis, however, results in the destruction of Tryptophan, and the deamidation of Asparagine and Glutamine into Aspartic acid and Glutamic acid, respectively, and does not allow a reliable quantification of Cystine/Cysteine, if not previously chemically modified (alkylated or oxidized) to a stable derivative.
The experimentally determined values may deviate to up to about 10% from the theoretical values. In case of hydrolyzed protein samples, a higher deviation may be found for the oxidation-sensitive amino acids (Serine, Threonine, Tyrosine and Methionine), the hydrophobic amino acids Valine, Isoleucine and Leucine (if only one hydrolysis time has been used**), due to difficult to hydrolyze hydrophobic-hydrophobic peptide bonds) as well as for values for alkylated cysteine. In general, the composition analysis of proteins should result in values that are within 20% of the theoretical values. If not, one should have serious doubts about the purity of the protein sample.
The experimental values determined for proteins present in a small piece of gel, may deviate somewhat more from the theoretical values. The highest accuracy will in this case be obtained, if a blanc piece of gel from the same SDS-PAGE is simultaneously analyzed for background correction.

For analysis of un-hydrolyzed samples, to quantify free amino acids, the same instrument and derivatization procedures are used. For such analyses, the above mentioned remarks concerning the consequences of acid hydrolysis, like destruction of Trp and de-amidation of Asn and Gln, are not valid. These latter amino acids can be well determined in un-hydrolyzed samples, as well as many other amino acid derivatives.

The chromatogram reports of the amino acid analysis have been checked and where necessary, corrections, additions and/or remarks have been made. The notes have been made on the original reports to avoid any possible copying errors. Also, a separate interpretation report ('Interpretation Amino Acid Analysis results') is enclosed. The calculation of the composition ('Comp.') of hydrolyzed samples is based on the molecular weight of the protein/peptide and/or on a certain (known or assumed) number of residues for one specific amino acid.

We would like to emphasize that the calculated amino acid composition and/or protein content is based on the best possible interpretation of the chromatograms obtained. However contamination of a sample, especially with (other) protein material and/or free amino acids, may lead to incorrect values (for instance, the calculated protein content of mixtures, if given, is 75-100% of the actual value).

***In case a reliable amino acid composition analysis should be obtained from proteins, several hydrolysis times should be performed in order to be able to calculate corrections for slowly hydrolyzing hydrophobic-hydrophobic amino acid peptide bonds on one hand, and to be able to correct as good as possible for destruction of oxidation-sensitive amino acids*. .

1. Schuster, R. (1988) *J. Chromatog.* **431**, 271-284
2. H. Brückner & T.Westhauser, (2003) Amino Acids **24**: 43–55