

Determination of optical purity of amino acids amino acid derivatives and peptides

We offer two different methods for determination of optical purity of the amino acids of peptides:

FID-method (for amino acids (A.0.1.0.) and amino acid derivatives (A.0.1.) and, if high standard deviation is acceptable, peptides(A.0.2.):

The first methods involves for peptides but also for some amino acid derivatives hydrolysis with 6N hydrochloric acid. The free amino acids are derivatized and gas chromatographically separated on a chiral column. This method however suffers from one weakness: under the condition of hydrolysis, racemization occurs and the amount of racemate determined represents the sum of the amount originally present in the peptide plus the generated during hydrolysis. The last can be estimated using a standard peptide and can be subtracted from the determined values. However the standard deviation is high due to matrix and sequence influences.

For **free amino acids** the method is appropriate because no hydrolysis is performed and no byproducts are to expect.

Also for most of the **amino acid derivates** the method is suitable. If however the cleavage leads to high concentration of byproduct or the sample itself is contaminated, co-elution with any contamination is possible and leads to incorrect results. Seldom racemization is observed during sample preparation. These possible problems are minimized by the GC-MS (A.0.3.) analysis.

For determination of enantiomeric purity of **peptides** the method can give an estimation if racemization is possible. However reliable determination <1%, for Cysteine <10% is not possible with this method.

What we need:

- 1mg of the sample.
- The amino acids which are to determine or better the sequence.
- Protective groups if present.
- Expected peptide content.

GC-MS method (A.0.3.):

Unambiguous quantitation of peptide epimers involves hydrolysis with 6N D₂O/DCl. The amino acids are derivatized using deuterated reagents. Racemization during this sample preparation is accompanied by deuterium exchange in the α -position (deuterium label). The proportion of D-amino acid originally present in the peptide is thus represented by the relative amounts of the unlabeled form which is monitored by mass spectrometry.

This method is also indicated if the enantiomeric purity of amino acid derivatives is needed with low standard deviation (eg. for derivatives with a specification <0.3%)

The limit of quantitation is 0.1% of the optical antipode.

The standard deviation is <0.1%.

What we need:

- 0.5 to 1mg of the sample (plus some sample for sample handling).
- The amino acids which are to determine or better the sequence.
- Protective groups if present.
- Expected content.

The following amino acids are not to consider as different amino acids:

Asn and Asp (determined as asp)

Gln and Glu (determined as glu)

Thr and allo Thr (all the 4 optical isomers are determined)

Ile and allo Ile (all the 4 optical isomers are determined)

If you need reliable results <1% respectively <10% for cysteine and the amino acid which is linked on Cys, peptides needs to be analyzed using this GC-MS method.



You will receive the invoice together with the results by mail. By fax you receive the results 4-5 days after receipt of the sample.

Terms of payment: 30 days net, all charges on your side.

Shipment:

To speed up custom regulation please take care that proforma invoice is less than 20US\$. In this case no additional custom fee will be invoiced.

Please contact us if you have any questions.

I would be pleased if we could serve you.