ELAB: One of the Most Potent Amino Acid Analysis

Introduction

The accuracy of an analysis is largely determined by its reproducibility. In quantitative chromatographic analysis there must be a reproducible correlation between the amount of substance and the measured result. For the sake of simplicity, this relationship should be linear.

Since most detectors do give a linear response within a reasonably wide range of concentrations, an analysis in which it could be ensured that 100% of the sample reached the detector would be unproblematic. The accuracy of the analysis would depend only on the accuracy of the integration and substance-specific response factors. In most analyses, however, this situation does not apply. Prior to detection, the sample must often be subjected to various procedures which may impair the accuracy of the result. These include:

- Clean up of the sample: this may result in non-reproducible losses or decomposition of individual substances.
- Derivatisation: incomplete reaction entails the danger of varying yields. There is also a risk of decomposition of individual substances or substance groups.
- Injection in the chromatographic system: the danger of discrimination is especially high in capillary gas chromatography. If split injection is used, control of discrimination of substances with high boiling points is imperative if the analysis is performed over a wide temperature range. Injection using a syringe can also result in discrimination for such substances [1]. On-column injection [2, 3] or "cold"- injection systems can reduce the problem.
- Separation of the sample in the chromatographic system: irreversible and reversible adsorptions distort the analytical result [4]. Sensitive substances or derivatives (e.g. TMS esters of carboxylic acids) can be decomposed by active sites in the chromatographic system.

Enantiomer-labeling [5]

If the losses mentioned above are reproducible, then they may be calculated and taken into account in the result. Generally this is not the case, especially when components from a complex matrix have to be determined.

This is where the internal standard method is used. A defined amount of a substance as ***chemically and physically similar as possible*** to the component to be determined is added to the sample, is co-analysed, and is used as a basis for the calculation. ***The greater the similarity between the chemical and physical properties of the standard and the component to be determined, the more similar will be the discrimination*** of the two substances in the analysis, and the better the analytical result. Often, more than one standard is used in order to achieve this. In addition, correction factors must be considered.

The method of enantiomer labeling uses a standard that admirably fulfils these requirements: the enantiomer is added to an optically active substance. In the case of amino acid analysis this is a mixture of all D-amino acids of defined composition. Each amino acid thus has its own standard - the enantiomer. The separation is performed on an optically active phase which separates all these components. This is possible in capillary gas chromatography using Chirasil-Val after derivatising amino acids to the n-propyl esters and acylation with trifluoroacetic anhydride.

The fact that ***the optical enantiomer is a standard with not just similar, but identical physical and chemical properties*** in an achiral pool gives the following advantages:

- if the standard is added directly after sample collection - e.g. immediately after collecting the blood sample for amino acid analysis in serum [6] - then the losses of standard and sample during clean up, derivatization and sample injection are identical because these processes are performed in an achiral medium.
- The determination of amino acids, even in complex matrices, is simplified considerably because the methods used in clean up may be such that quantitative recovery of the amino acids is not necessary.
- Substance-specific calibration factors need not be considered.

The only possible sources of error remaining are due to the integration and inaccuracy in adding the D-amino acid standard.
If the sample contains some proportions of the enantiomers used as standard, which is possible in the case of synthetic peptides, then these are determined in an analysis without addition of standard and taken into account in the quantitative calculation. The proportions of the L-form in the D-amino acid of the standard are also taken into account. For the commercially available standard these values are defined.

$$x_a = \frac{A_L - A_D * C_D + A_L * C_L - A_D * C_L * C_D}{A_D - A_L * C_L + A_D * C_D - A_L * C_L * C_D} m_D$$

Where A_L = peak area of L-enantiomer after addition of standard
A_D = peak area of D-enantiomer after addition of standard
C_L = D- : L-enantiomer before addition of standard
C_D = L- : D-enantiomer of standard added
m_D = amount of standard added
x_a = amount of amino acid being determined

The accuracy of the total analysis including sample preparation is more important than the accuracy of the chromatographic analysis alone. If, for example, hydrolysis or clean up is necessary, then the error arising from sample preparation is probably higher than the error incurred in the chromatographic analysis itself. Using the method of enantiomer labeling the standard deviation is notably low despite these steps. Even with amino acids known to decompose during hydrolysis (serine, tryptophan, and methionine) the standard deviation amounts to only about 5%. Only for cysteine is the error larger, since racemization during the hydrolysis is poorly reproducible.

The advantages of this method of analysis may be summarized as follows:

- ✓ High accuracy of the analysis even after extensive sample preparation. Even Serine, Methionine and Tryptophan is determined after acid hydrolysis with high accuracy.
- ✓ Standards are calibrated using standards from National Institute of standards and Technology (NIST, USA)
- ✓ In addition to quantitation of the amino acids, accurate information as so the optical purity of the sample is provided automatically.
- ✓ Possibility of using a large number of methods for sample preparation without affecting the accuracy of the result.
- ✓ The multitude of GC-detectors may be used, including MS coupling [7, 8].

References

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