INTERLABOR
BELP AG

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FACTSHEET

Nitrogen determination in proteins according to Kjeldahl

Introduction

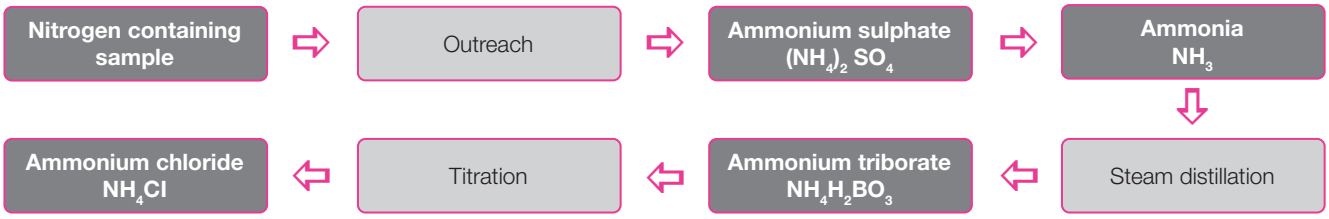
The Kjehldahl method for the quantitative determination of nitrogen content was published by the chemist of the same name as early as 1883.[1]

The method allows the determination of nitrogen in a large number of different nitrogen-containing matrices. Due to its versatility and high precision, it is used in a wide variety of applications. For example, the method is used in the testing of food and feed as well as environmental samples or pharmaceuticals.

Functional principle

In principle, the method consists of three steps. First, the sample to be analyzed is digested with sulfuric acid (step 1). The nitrogen contained in the sample is thus converted into ammonium sulfate which is then converted into ammonia by the addition of sodium hydroxide. Next, a steam distillation is carried out (step 2). This allows the ammonia and with it the nitrogen originally contained in the sample to be transferred from the digestion to a titration solution. After the titration has been completed (step 3, graph 1), the amount of nitrogen can be calculated on the basis of the amount of titration solution consumed.

Graph 1: Nitrogen determination according to Kjeldahl



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Nitrogen determination according to Kjeldahl within the scope of GMP

In order to be able to carry out the analysis within the framework of GMP, a product-specific validation is necessary. The validation process for the Kjeldahl method is demanding and requires high analytical care. In particular, the following key aspects must be taken into account:

- **Complete digestion of the sample material**
  - ⇨ **Control by linearity or digestion time possible**
- **Absorption of all nitrogen during distillation**
  - ⇨ **Control by linearity or distillation time possible**
- **Titration to the correct colour**
  - ⇨ **Control by repeatability* possible**

*Repeat precision is defined as relative standard deviation and is ≤ 2.0% for regular and ≤ 3.0% for laboratory precision.

In addition, a preliminary test is carried out to ensure that the digestion time or distillation time is sufficient to exclude the influence of minor fluctuations in the sample weight on the quality of the analysis.

After completion of the preliminary test, the following parameters are validated in the actual validation:

- **Selectivity (by means of blank value)**
- **Linearity (80%-120%)**
- **Repeatability (*n*=6) and laboratory precision (*n*=2 x 6)**
- **Accuracy in combination with precision (3x80%, 6x100%, 3x120%)**
- **Working range (80%-120%)**
- **Robustness (includes variation of digestion/ distillation time and concentration of the sample solution which is tested by linearity)**

Key data nitrogen determination according to Kjeldahl at Interlabor Belp AG

Nitrogen determination according to Kjeldahl as per Ph.Eur. can be offered under the following conditions:

- Quality of analysis: ISO 17025 or GMP
- Processing time: 15 working days from receipt of sample
- Analysis price for routine analysis: 540 CHF per sample

Depending on the project, different analysis qualities (ISO 17025 or GMP) and processing times (method development and validation about 8-12 weeks) can be offered. We would be happy to advise you in a personal meeting.

References

1. J. Kjeldahl, Neue Methode zur Bestimmung des Stickstoffs in organischen Körper, Zeitschrift für Analytische Chemie, 22(1)/1883, S. 366–382.

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