# best@buchi no. 65
## Decode the mysteries of the Kjeldahl Tablets

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## Abstract

This best@buchi presents the BUCHI assortment of Kjeldahl Tablets and explains the effects of their different components and compositions. Every component has a special function. Copper sulfate and titanium dioxide possess catalytical effects. Potassium and sodium sulfate increase the boiling point of sulfuric acid and furthermore have a hygroscopic effect. Silicone acts as an antifoam agent. The various combinations of these different components make it possible to meet the manifold demands of the Kjeldahl digestion [1]. Different samples (waste water, milk and meat) as well as different reference substances (urea, tryptophan and glycine) were used in order to be able to compare the performance of the various Kjeldahl Tablets [2, 3, 4].

## History of the Kjeldahl method

The Danish chemist Johan Kjeldahl published his method for nitrogen and protein determination in 1883 [5]. In order to determine the accurate nitrogen content in a sample, he developed a method which involves a three-step procedure: In a first step the sample is digested together with sulfuric acid and a metal catalyst. In this step the organic bounded nitrogen is converted into ammonia. After neutralization, the ammonia is then, in a second step, separated by means of steamdistillation and trapped into a receiving solution. In the third step the ammonia is titrated in order to determine the nitrogen content indirectly.

In Kjeldahl's original publication, dry potassium permanganate powder was added as a catalyst. This method is still universally accepted for nitrogen determination and it has also been the reference method (ISO, AOAC) for determining the protein content for many years. Although other methods claim to be faster and more specific, none of them can cope with the variety of sample sizes or compositions than Johan Kjeldahl's method.

While the principle of the method remains unchanged, various modifications have improved the modern Kjeldahl digestion.

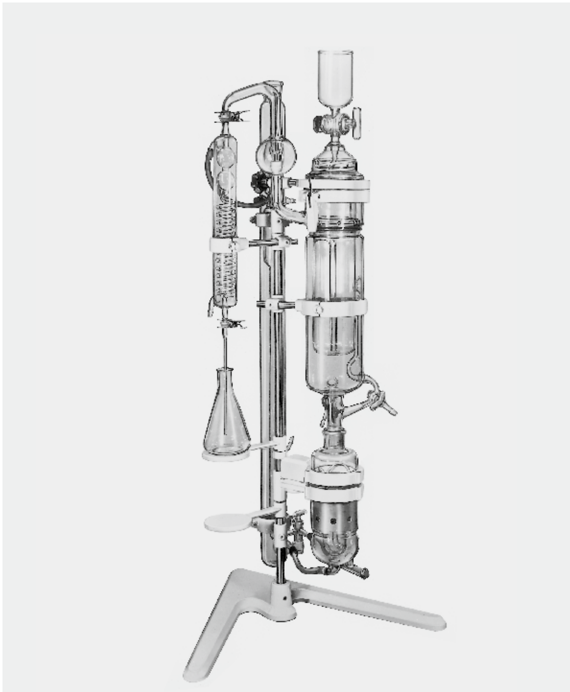


*Figure 1: First BUCHI Kjeldahl distillation unit*

## Today's needs

Recently, the problems of environmental pollution, water quality requirements and user safety have become increasingly important. Moreover increasing sample loads in combination with limited manpower ask for a fast handling procedure and process. Hence, a compromise between ecological and economical aspects must be found. Another crucial factor is the conformity to official regulations like AOAC and ISO.

## Components

Usually the Kjeldahl Tablets consist mainly of an inert salt ($K_2SO_4$ or $Na_2SO_4$) which increases the boiling point of sulfuric acid. A further component is 1 – 3 % of one or several metal catalysts which speed up the chemical reaction. Some samples additionally require additives like silicone to reduce the formation of foam at the beginning of the digestion.

## Catalysts

Different reports show that a various number of catalysts help to accelerate the digestion process and to increase the recoveries [6, 7]. Typically mercury, selenium, titanium and copper are the catalysts of choice to help increase the efficiency and speed of the digestion process. However, mercury and selenium are highly toxic and should rather be avoided. It is therefore advisable to use titanium and copper which are of low toxicity but almost as effective [8].

Advantages and disadvantages of the most common catalysts:

| Catalyst | Advantage | Disadvantage |
|---|---|---|
| Mercury | very short digestion time at reproducible results | toxic and expensive, waste must be collected for safe disposal |
| Selenium | very short digestion time, even for fatty samples | very toxic, N losses can occur when incorrect ratio, too long digestion times or too high temperatures are applied |
| Copper | compliant to official regulations, low toxicity | slower digestion process than selenium and mercury |
| Titanium | low toxicity and better recovery than copper | slower digestion proccess than mercury |

## The digestion temperature

The rate speed of a chemical reaction also depends on the temperature [9]. A useful generalization implies that the reaction rate doubles with every 10 °C increase in temperature. It is however not possible to heat the digestion mixture above its boiling point by only increasing the heating temperature.

**1883 J. Kjeldahl published the nitrogen determination method** „The principle of the new method is to heat the test material for some time with a large quantity of concentrated sulfuric acid at a temperature close to the boiling point and to oxidize the solution thus obtained with an excess of dry potassium permanganate powder…“

To increase the boiling point of sulfuric acid (approx. 335 °C) it is necessary to add inert salts. Different types of inert salts (potassium vs. sodium sulfate) increase the boiling point as follows:

- 354.1 °C → $H_2SO_4$ + potassium sulfate
- 351.8 °C → $H_2SO_4$ + potassium and sodium sulfate
- 348.0 °C → $H_2SO_4$ + sodium sulfate

Potassium sulfate increases the boiling point of the digestion mixture by around 6 °C more than sodium sulfate. During the digestion process the sulfuric acid evaporates thereby changing the salt / acid ratio and leading to even higher temperatures (see Figure 2). The boiling temperature should never exceed 380 °C as at a temperature of 390 °C or higher nitrogen losses can occur due to a possible transformation of ammonia into elemental nitrogen gas ($N_2$).

Generally it can be said that potassium sulfate is the most adequate salt to be used for a Kjeldahl digestion. This is due to the fact that it achieves a higher boiling point, and, moreover to the circumstance that mixtures with sodium sulfate rather tend to crystallize.

**Temperature measurement in digestion mixtures**

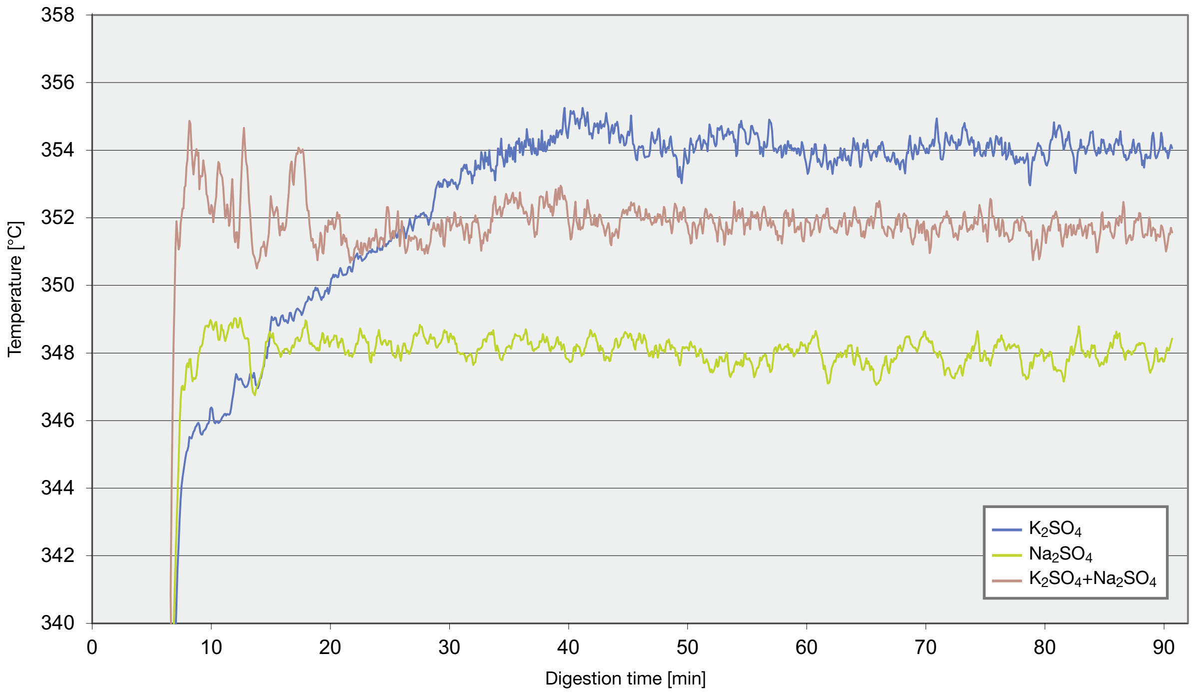



*Figure 2: Temperature measurement in different digestion mixtures*

## The ideal composition

As mentioned above the perfect Kjeldahl Tablet offers an ideal balance between different criteria such as easy handling, environmental effects and digestion speed.

Due to its easy and unproblematic handling copper sulfate outmatches mercury and selenium as the ideal catalyst. Even when used in only small amounts it is the main component which help to speed up digestions
→ *Due to its low toxicity but nevertheless very effective catalytic effect, copper sulfate nowadays often replaces selenium and mercury. Copper sulfate is the most frequently used catalyst, and is also mentioned in modern regulations of AOAC and ISO.*

Titanium dioxide also has catalytical effects and helps to increase the digestion speed. For highly organic samples titanium dioxide shows better effects than copper and it is also accepted by AOAC and ISO.
→ *The shortest digestion times can be achieved using the catalysts titanium dioxide and copper sulfate in a mixing ration of 1:1.*

Potassium and sodium sulfate increase the boiling point of sulfuric acid and therefore speed up the digestion
→ *Potassium sulfate achieves a higher boiling point than sodium sulfate and moreover decreases the risk of crystallization. Therefore potassium sulfate is the most adequate inert salt for a Kjeldahl digestion.*

## The BUCHI Tablets

BUCHI offers six different types of tablets: "Titanium", "Missouri" and "ECO" are made for the standard Kjeldahl applications. The Tablets, "Titanium Micro" and "Copper Micro" are generally used for micro-Kjeldahl applications. In case of foam formation the additive Tablet "Antifoam" can be used as a general purpose foam suppressant. It does not contain a metal catalyst [10].

*Figure 3: BUCHI Kjeldahl Tablets*

The three different Kjeldahl Tablets for standard applications (Titanium, Missouri, ECO) were tested for three typical samples namely milk, meat and waste water. These samples were selected because of their broad spectrum of nitrogen contents from very low (5 mg N/L) to high (18 %) and because of the very different sample matrices (org. matter and water). Comparative experiments were performed in order to determine the effect of the different Kjeldahl Tablets (Titanium, Missouri and ECO) on different sample matrices. The ideal amount of sulfuric acid used for the experiment depends on the weight of the Kjeldahl Tablets. Optimal conditions can be achieved with a ratio of 2:1 (2 mL $H_2SO_4$ to 1 g of Kjeldahl Tablet). The digestion time was varied in the experiments in order to be able to see the advance of each type of Kjeldahl Tablet.

| | | Inert salt | Metal catalyst | | Ideal conditions |
|---|---|---|---|---|---|
| Type for standard Kjeldahl applications | Tablet weight [g] | $K_2SO_4$ [%] | $CuSO_4 \times 5\ H_2O$ [%] | $TiO_2$ [%] | Amount of $H_2SO_4$ (ratio 2:1) for 2 tabs [mL] |
| Titanium | 3.71 | 94.3 | 2.8 | 2.8 | 15 |
| Missouri | 5 | 99.6 | 0.4 | --- | 20 |
| ECO | 4 | 99.94 | 0.06 | --- | 16 |

| | | Inert salt | Metal catalyst | | Ideal conditions |
|---|---|---|---|---|---|
| Type for micro-Kjeldahl applications | Tablet weight [g] | $K_2SO_4$ [%] | $CuSO_4 \times 5\ H_2O$ [%] | $TiO_2$ [%] | Amount of $H_2SO_4$ (ratio 2:1) for 1 tab [mL] |
| Titanium Micro | 1.59 | 94.3 | 2.8 | 2.8 | 3 |
| Copper Micro | 1.65 | 90.0 | 10.0 | --- | 3 |

| | | Inert salt | Antifoam agent | Ideal conditions |
|---|---|---|---|---|
| Type for foaming samples | Tablet weight [g] | $Na_2SO_4$ [%] | Silicone [%] | |
| Antifoam | 1 | 97.0 | 3.0 | one tab per sample tube |

Reference experiments:

1. Milk samples and the corresponding reference material tryptophan
2. Meat samples and the reference substance glycine
3. Waste water (TKN) and urea stock solution

All digestions were performed with the SpeedDigester K-439 and analized with the new KjelMaster System K- 375 / K-376

The ideal volume of sulfuric acid depends on the type of Kjeldahl Tablet and on the weight of the sample. Usually, two tablets are used, and therefore the volume of sulfuric acid can vary from 20 mL with the Tablet Missouri to 15 mL with the Tablet Titanium.

*Figure 4: Fully automatic KjelMaster System K-375/K-376*

*Figure 5: SpeedDigester K-439 with 500 mL tubes*

### 1. Determination of nitrogen and protein in milk [2]

According to ISO 8968 tryptophan or lysine hydrochloride is used as a reference substance [11, 12, 13]. With help of tryptophan a good differentiation between the effects of the individual Kjeldahl Tablets can be achieved as it contains an aromatic heterocyclic functional group which is difficult to digest. Tryptophan is an essential amino acid that is a constituent of proteins in food.

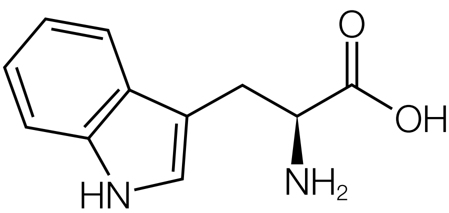



*Figure 6: Tryptophan*

After a digestion time of 85 min, the Tablet Titanium is the only catalyst which shows a recovery (98.8 %; rsd 0.4 %) within the specification of > 98 % [11]. The Tablets Missouri and ECO require an additional 30 min to reach the specification (Figure 7).

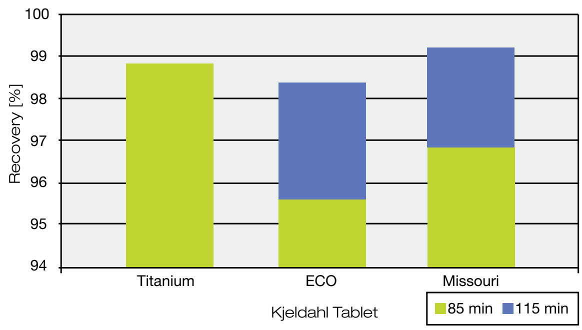



*Figure 7: Recovery (%) of tryptophan n=3, digested with different types of Kjeldahl Tablets and using different digestion times*

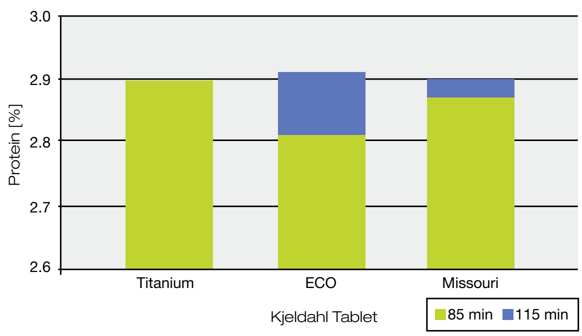



*Figure 8: Protein content (%) of strawberry milk drink (n=3) using different Kjeldahl Tablets and digestion times*

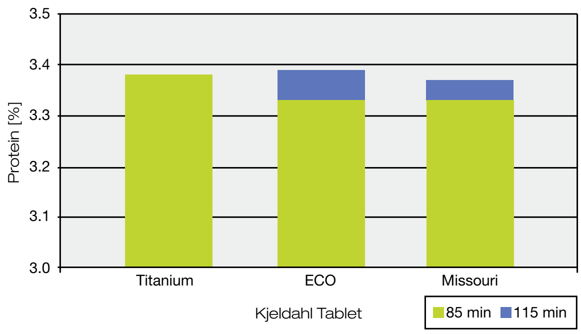



*Figure 9: Protein content (%) of whole milk (n=3) using different Kjeldahl Tablets and digestion times*

Figures 8 and 9 present the behavior of the Kjeldahl Tablets during the experiments with the sample materials strawberry milk drink and whole milk. The results are similar to the ones obtained in the experiments with tryptophan.

Yet again Titanium is the only catalyst which is able to digest the samples in 85 min. The catalyst ECO and Missouri require an additional 30 min to complete the digestion. It is possible that the combination of copper and titanium helps to digest the ring-bonded nitrogen faster, than copper on its own. In the official regulation AOAC 991.20, a combination of copper sulfate and potassium sulfate, similar to the one which can be found in the catalyst Missouri, is described.

The comparison of the three catalysts proves that every type of Tablet has its own benefit. All three catalysts are able to digest tryptophan and milk.

If the speed of the digestion is crucial, for example in laboratories with a high sample throughput, Titanium would be the best Tablet. If the main importance lies on working in full compliance with official regulations [11, 12, 13], the Tablet Missouri fulfills the needs best.

The Tablet ECO digests as fast as the catalyst Missouri. Nevertheless ECO has a lower content of copper sulfate, which makes it more environmentally friendly.

## 2. Determination of nitrogen and protein in meat products [3]

No major differences between the individual catalysts could be determined during the experiments in which glycine was used as a reference material. When taking a closer look at the structure of the amino acid, one detects a very simple composition. This makes it easy to digest.

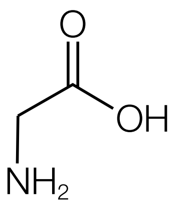



*Figure 10: Glycine*

All recoveries after varying the total digestion times and after using the different Kjeldahl Tablet types were within the specification of 98 – 102 % [17].

When analyzing real sample materials with different contents of fat, like for example smoked turkey or salami (Figure 11 and 12), a significant difference between the three examined Kjedahl Tablets becomes obvious. Smoked turkey has a fat content of only 2 % in contrary to salami with a fat content of 32 %.

The Kjeldahl Tablet Titanium can complete a digestion within 90 min. This Tablet is the only catalyst that contains titanium(IV)oxide. Moreover it has the highest content of copper sulfate of all described Kjeldahl Tablets. The Kjeldahl Tablet Missouri features the second highest content of copper sulfate. It takes this catalyst a total time of 120 min to accomplish the digestion. The catalyst Missouri is mentioned in some official methods for determining protein contents in meat [14, 15].

The content of copper sulfate in the catalyst ECO has been reduced to a minimum. Nevertheless ECO's overall digestion time is comparable to the one achieved with the Missouri Tablet. The experiments prove that samples containing a high amount of organic matter require a higher quantity of catalytically active components such as copper sulfate or titanium(IV)oxide in order to speed up the digestion. The following figures 11 to 12 summarize the most prominent advantages of each catalyst.

Same as for the sample matrix milk, Titanium also proves to be the ideal catalyst for laboratories with a high sample throughput, when it comes to the sample matrix meat. Likewise, the catalyst Missouri is described in the regulation for the digestion of meat [14] and must therefore be selected. Despite the lower content of copper sulfate, the catalyst ECO can also be used for digesting meat samples and glycine although it requires a longer digestion time.

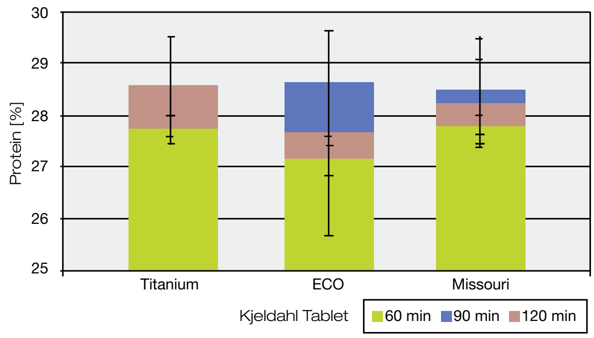



*Figure 11: Protein content (%) of smoked turkey after using different types of Kjeldahl Tablets and varying digestion times. The error bars represent the RSD (n=3)*

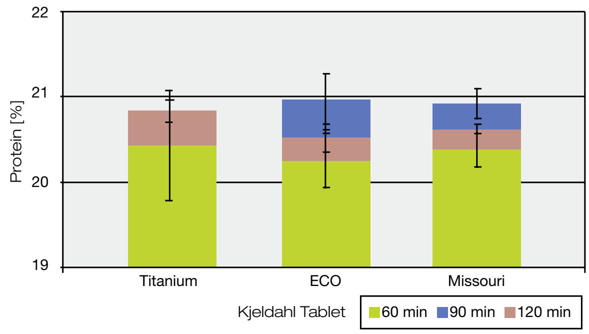



*Figure 12: Protein content (%) of salami after using different types of Kjeldahl Tablets and varying digestion times. The error bars represent the RSD (n=3)*

## 3. Determination of total Kjeldahl nitrogen (TKN) in water [4]

In accordance with the US EPA Method 351.3, urea was selected to sample an organic nitrogen-containing compound of biological origin. Three different TKN concentrations were used in order to compare the different Kjeldahl Tablets. The results are presented in Figure 13.

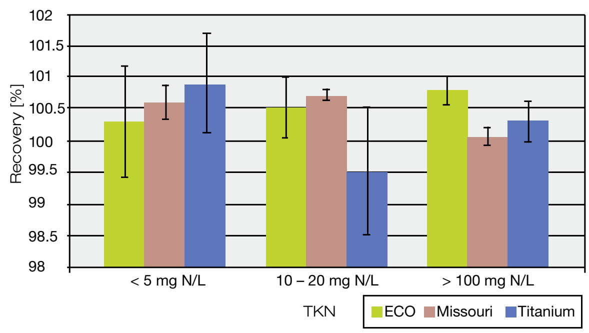



*Figure 13: Recoveries (%) of different TKN concentrations using different Kjeldahl Tablets. The error bars represent the RSD (n=3).*

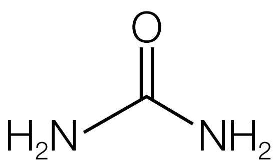



*Figure 14: Urea*

All recoveries are within the required specification of 98 – 102 % [17]. All three types of catalyst require the same digestion time, which depends on the sample volume. Due to this fact the election criteria for using any of the three Kjeldahl Tablets cannot be based on speed but must rather depend on eco-friendliness and chemical reduction.

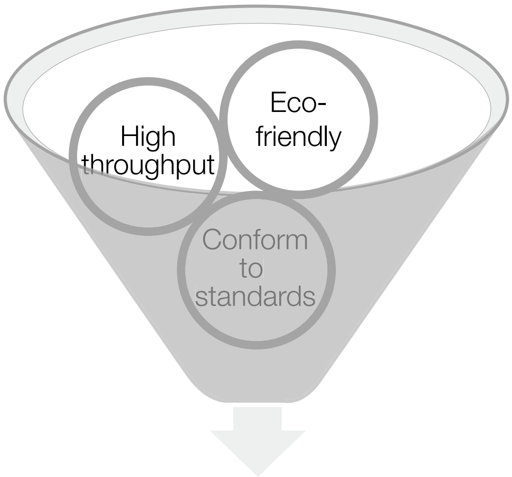
The Kjeldahl Tablets ECO (4 g) and Titanium (3.7 g), which weigh less than the Tablet Missouri (5 g), only require 8 mL of sulfuric acid in order to reach the optimal ratio (1:2) of salt to sulfuric acid.

A closer look to the composition of the catalysts reveals that ECO contains the lowest copper content of all three types of Kjeldahl Tablets and is therefore the most eco-friendly choice for digesting water and waste water samples.

## Selecting the appropriate Kjeldahl Tablet

The decisive factors for selecting a particular catalyst follow ecological, economical and practical aspects like for example reducing the formation of foam. The Tablet Titanium is the most balanced compromise in terms of speed and environmental concern. If environmental aspects are of major importance or the to-be analyzed sample only contains very little organic material, the Kjeldahl Tablet ECO might be the ideal choice as it features the lowest content of copper.

If the main incitement is to reduce the amount of chemicals used in the process, and therefore also the running costs, it is advisable to prefer the micro-Kjeldahl method and the corresponding micro Kjeldahl Tablets (Copper Micro or Titanium Micro).




| Tablet type | Tablet weight [g] | Recommended sample type | Benefit |
|---|---|---|---|
| Titanium | 3.71 | High in organic matter, high fat content | · Time saving<br>· Mentioned in official regulations<br>· Reduced $H_2SO_4$ amount (15 mL) due to the low Tablet weight |
| Missouri | 5.00 | Middle or high in organic matter | · Compromise between Titanium and ECO<br>· Mentioned in official regulations |
| ECO | 4.00 | Low in organic matter | · Most eco-friendly Tablet due to the very low copper content<br>· Reduced $H_2SO_4$ amount (16 mL) due to the low Tablet weight |

## Conclusion

Each type of BUCHI Kjeldahl Tablet fulfills specific needs and has its very own advantage. It is therefore advisable to consider the individual and personal demands and requirements and selecting the Kjeldahl Tablet which meets these best. The handy tablet format simplifies the dosage of the catalyst, thereby minimizing error sources and creating ideal digestion conditions. The different weights of the Tablets allow adapting the amount of chemicals. The running costs for the determination can thereby be reduced. Harmful chemicals with toxic or polluting effects such as mercury or selenium are no longer part of the composition. The BUCHI Kjeldahl Tablets are therefore considerably safe for both humans and environment.

## Helpful tools

Find detailed information including our reference applications in the application database, the Kjeldahl Practice Guide for daily routine with several hints and general rules, the Kjeldahl Guide for detailed background information as well as different helpful apps.
www.buchi.com/kjeldahl/applications/literature

Find the optimal Kjeldahl Tablet matching your demands with the KjelTab app and the ideal setting for your method with the KjelCalc app for smartphones and tablet PC's on www.buchi.com/kjeldahl/apps.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