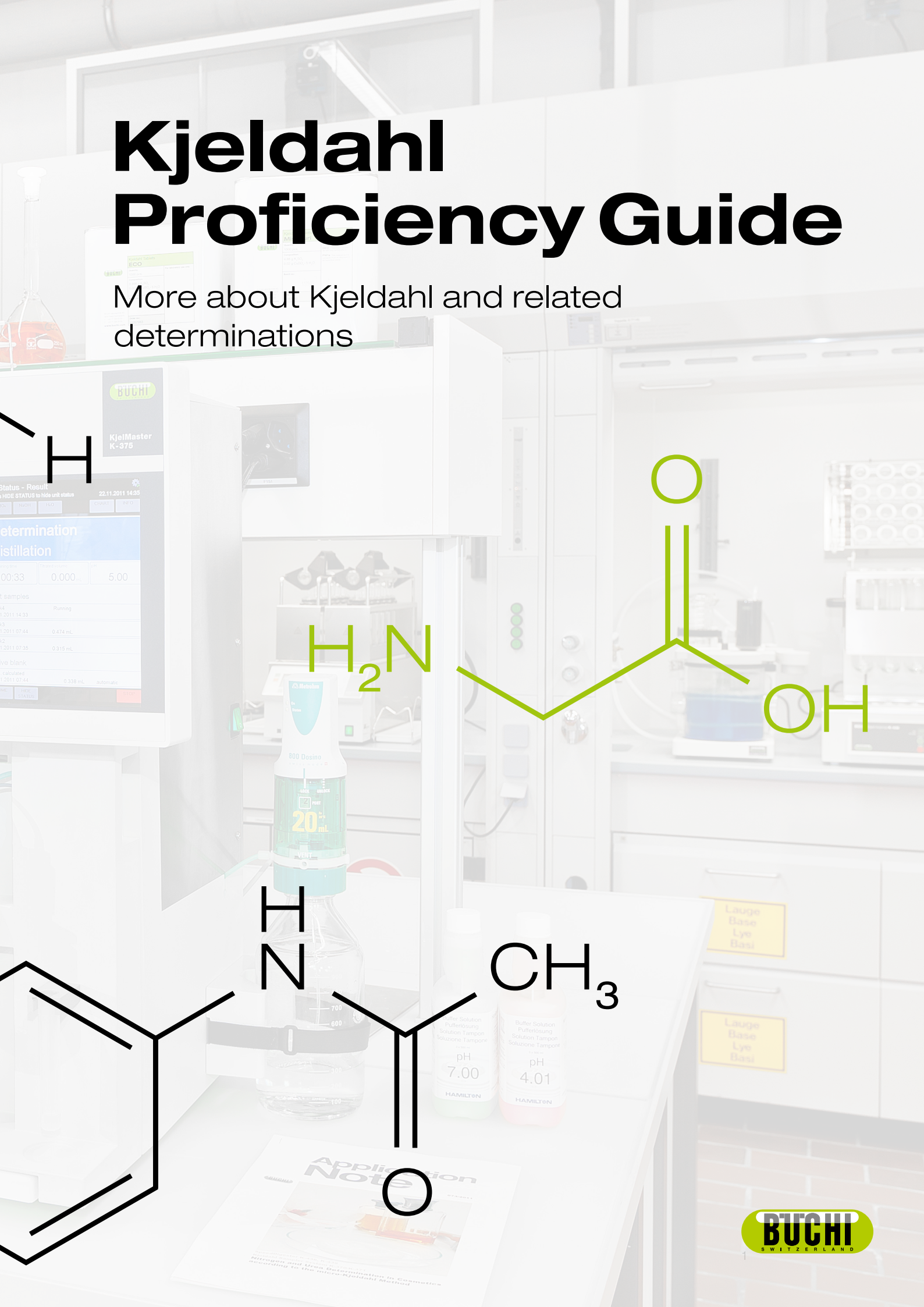
Kjeldahl Proficiency Guide

More about Kjeldahl and related determinations



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Foreword

Over 50 years of experience developing Kjeldahl instrumentation and applications are summarized in this Guide. The aim of it is to share the BUCHI expertise in regards to the nitrogen and protein determination according to the Kjeldahl method. The deeper understanding of the procedure will help laboratory personnel, laboratory supervisors, students and teachers to optimize the individual method and increase the reliability of the results.

It is our intention to revive the basic knowledge needed to understand the chemical and physical background associated with nitrogen determinations according to Kjeldahl and provide clear instructions in a wide area of Kjeldahl applications. The mayor theoretical part of the *Kjeldahl Knowledge Base* contains basic knowledge, consolidation of the Kjeldahl know-how followed by an extensive list of regulations and actual BUCHI Application Notes describing successful nitrogen determinations.

The comprehensive *Kjeldahl Proficiency Guide* builds on the insights in the *Kjeldahl Knowledge Base* and explains them in greater depth. Theoretical and practical aspects of nitrogen and protein determination according to Kjeldahl and related analyses are presented and explained in detail.

The *Kjeldahl Proficiency Guide* aims to help you understand the chemical basis for various nitrogen determination processes and to carry them out.

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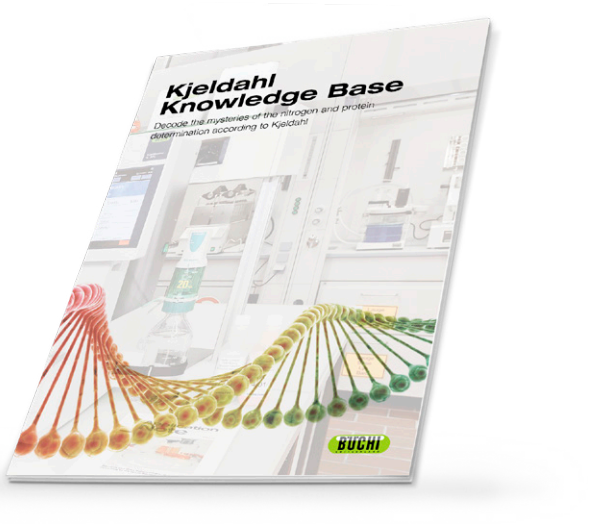
1. Introduction

BUCHI has offered «Quality in your hands» since 1939

The second installment of the *Kjeldahl Knowledge Base* BUCHI aims to support daily work with high-quality laboratory equipment and theoretical background knowledge. This Guide is intended to provide greater depth and understanding of the Kjeldahl process and other related nitrogen determination processes and applications.



Kjeldahl Knowledge Base
Over 50 years of experience are summarized in this Knowledge Base.
www.buchi.com/en/applications/literature

Application Finder
Look up detailed information on a large number of sample matrices.
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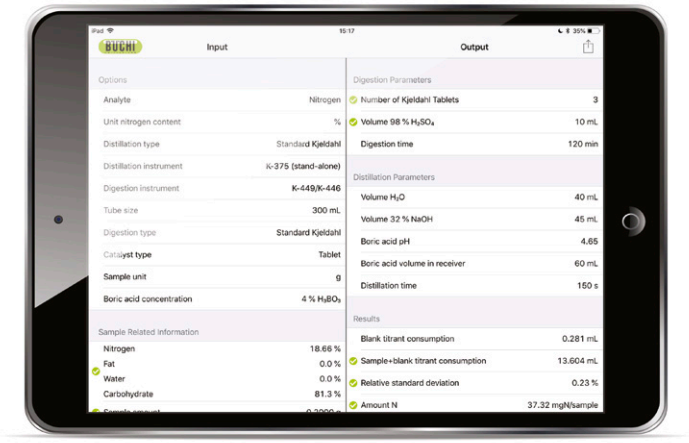

KjelOptimizer App
Optimize individual Kjeldahl methods.
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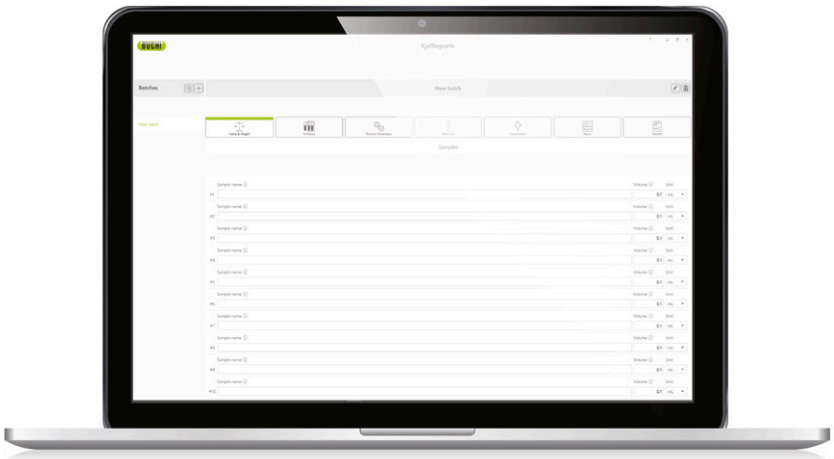

KjelReports App
Easily calculate results and produce reports for Kjeldahl and SO_2 determinations.
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STOP

2. Optimizing

the Kjeldahl process

1. Digestion
2. Distillation
3. Recovery rate
4. LOD & LOQ

Kjeldahl Proficiency Guide
Optimizing

This chapter looks in detail at how to optimize the Kjeldahl process with regard to digestion and distillation. BUCHI instrumentation and technology can help to reduce the processing time for nitrogen and protein determination according to Kjeldahl. In addition, process reliability, economic and ecological aspects are taken into account.

2.1 Optimizing the digestion process

The digestion time depends on the sample matrix and the following factors: volume of sulphuric acid, ratio of acid to sulphate salts, catalyst type, digestion temperature and optional addition of hydrogen peroxide.

Dependence on chemical structure

Samples containing aromatic structures require more time to become fully digested. The table below depicts the transparent (clearing times) for the digestion solution and the total digestion times for urea, glycine, tryptophan and acetanilide. In addition, information on the experimental recovery rate and optional addition of hydrogen peroxide is provided. Aromatic tryptophan shows the extended digestion time with resonance-stabilized nitrogen.

Correlation of times within which samples become transparent (clearing times) and total digestion time showing recovery rate using Kjeldahl titanium tablets

Compound	Clearing time [min]	Digestion time [min]	H_2O_2 addition	Recovery [%]	Reference: Application Note
Urea	11	70	No	99.8	AN124/2013
Glycine	15	110	No	100.0	AN114/2013
Glycine	–	65	Yes	99.4	AN115/2013
Tryptophan	16	120	No	98.8	AN102/2013
Tryptophan	–	60	Yes	98.8	AN103/2013
Acetanilide	–	65	Yes	100.5	AN107/2013
Acetanilide	–	135	No	99.1	AN108/2013

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Dependence on sulphuric acid volume

Samples that consist of fat or contain a high proportion of fat, oil or carbohydrates have a high foaming potential while carbonizing. Increasing the volume of sulphuric acid enables decomposition of the carbonized material and reduces the formation of hydrocarbon fumes. If a sample with a low nitrogen content is analysed, the sample quantities must be increased sufficiently for a determinable quantity of nitrogen to be present in the sample tube. Large sample quantities, on the other hand, require a larger quantity of sulphuric acid to enable complete digestion.

Dependence on the ratio of sulphuric acid to sulphate salts

With samples that require larger quantities of sulphuric acid, the ratio of sulphuric acid to sulphates must be kept at a minimum to maintain the optimum boiling temperature. For more information please read the *Kjeldahl Knowledge Base*.

Dependence on catalyst type

As described in the *Kjeldahl Knowledge Base*, there are various types of catalysts available for specific types of sample. Fatty, oily and heterocyclic aromatic compounds are more easily digested if the catalyst contains selenium or mercury. However, mercury and selenium are highly toxic and should be avoided. Therefore, it is advisable to use titanium and copper, which have low toxicity but are comparable in their effectiveness.

The digestion conditions can be further optimized by the use of anti-foaming agents, boiling rods and the implementation of temperature gradients.

Kjeldahl Tablet Configurator
Use the configurator to select the Kjeldahl Tablet for the digestion that suits your needs best.
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Decode the mysteries of the Kjeldahl Tablets.
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Liquid samples

Large quantities of liquid such as are used for determination of the total Kjeldahl nitrogen (TKN) require the use of boiling rods. They allow boiling delays to be avoided during digestion. Boiling chips are not recommended because they can cause problems with distillation equipment by blocking hoses or damaging valves and pumps. Anti-foaming agents reduce the surface tension and the viscosity of samples, which facilitates the release of gases from the foam. Examples of frequently used anti-foaming agents are Antifoam Kjeldahl tablets, a spatula tipful of stearic acid, silicone oil and hydrogen peroxide. As a rule, those substances are sufficient to suppress foaming. The amount of foam produced is proportional to the sample size. Therefore, foaming can be minimized by reducing the sample size. Alternately, using larger sample tubes – say, 500 mL instead of 300 mL – can also reduce the problem of foaming samples. Critical samples can also be pretreated with hydrogen peroxide or pre-digested at room temperature with hydrogen peroxide.

BÜCHI boiling rods

BÜCHI 300 mL sample tube

BÜCHI 500 mL sample tube

Process acceleration by hydrogen peroxide

Digestion processes can be accelerated by means of hydrogen peroxide (H_2O_2). When doing so, the catalyst can either be completely replaced by H_2O_2 or a combination of catalyst and H_2O_2 can be used. There is a long history of hydrogen peroxide use for Kjeldahl digestion, but it is now rarely chosen as most of the standards describe only catalyst mixtures. The introduction of the SpeedDigester series saw the development of new ideas regarding the use of hydrogen peroxide in the digestion process and they are documented in the BUCHI application notes. Milk [1] and milk products [2] have been used as the sample matrices for accelerated protein determination with H_2O_2 and the classic process. For protein determination in meat products the process has been carried out both using H_2O_2 [3] and using Kjeldahl tablets in combination with H_2O_2 [4].

The three different digestion methods for protein determination have been demonstrated using tofu [5] and milk as protein matrix and compared. The comparison can be retrieved in the BUCHI Application Finder.

[1] BUCHI Application Note No. 054/2010, Nitrogen and Protein Determination in Milk by Digestion with Hydrogen Peroxide and Sulfuric Acid

[2] BUCHI Application Note No. 102/2013, Nitrogen and Protein Determination in Dairy Products according to the Kjeldahl Method

[3] BUCHI Application Note No. 071/2011, Nitrogen and Protein Determination in Meat Products by Accelerated Digestion with Hydrogen Peroxide and Sulfuric Acid

[4] BUCHI Application Note No. 115/2013, Accelerated Nitrogen and Protein Determination in Meat Products with Kjeldahl Tablets and Hydrogen Peroxide

[5] BUCHI Application Note No. 155/2016, Three digestion methods for protein determination in tofu

Comparison of the three different digestion methods for protein determination in milk according to Kjeldahl

Protein determination in milk	Digestion with catalyst	Digestion with H_2O_2	Digestion with H_2O_2 + catalyst
	AN 102/2013	AN 054/2010	AN 103/2013
Sample size	3 g	5 g	2 g
Sulphuric acid (98 %)	15 mL	20 mL	10 mL
Catalyst	7.5 g	No	3.71 g
H_2O_2 (30 %)	No	15 mL (twice)	8 mL

Temperature profile

Digestion unit	KjelDigester	SpeedDigester	KjelDigester
Preheating	300 °C	450 °C	330 °C
Step 1	340 °C for 15 min	450 °C for 20 min	420 °C for 60 min
Step 2	420 °C for 105 min	480 °C for 10 min	–
Total time	120 min	30 min	60 min

Application Finder
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Advantages and disadvantages

	Digestion with catalyst	Digestion with H_2O_2	Digestion with H_2O_2 + catalyst
Advantages	· Officially approved · Commonly used · User does not need to be in attendance	· Extremely fast · Eco-friendly	· Officially approved · User does not need to be in attendance
Disadvantages	· Time-consuming	· No official standards · Special suction module · User must be in attendance during digestion	· User protection shield required

Device

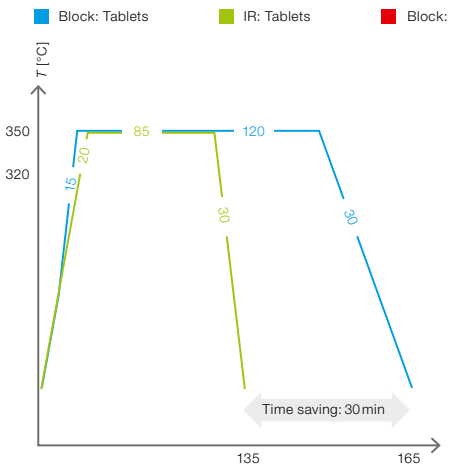
BlockDigester

BlockDigester

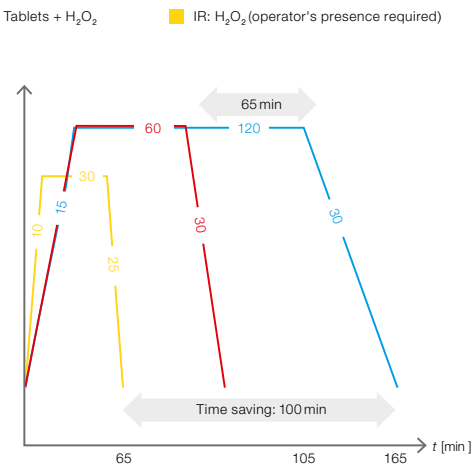
SpeedDigester

SpeedDigester

Reduce heat-up / cool-down periods

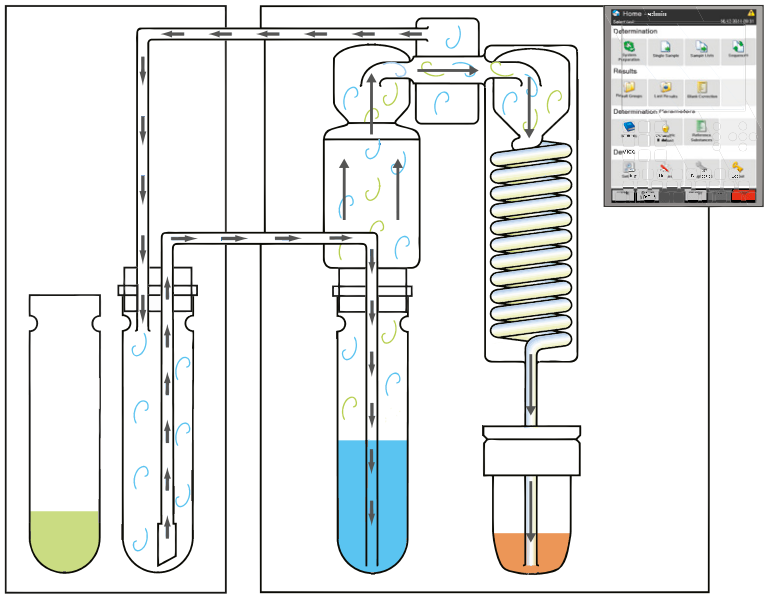


Reduce digestion period

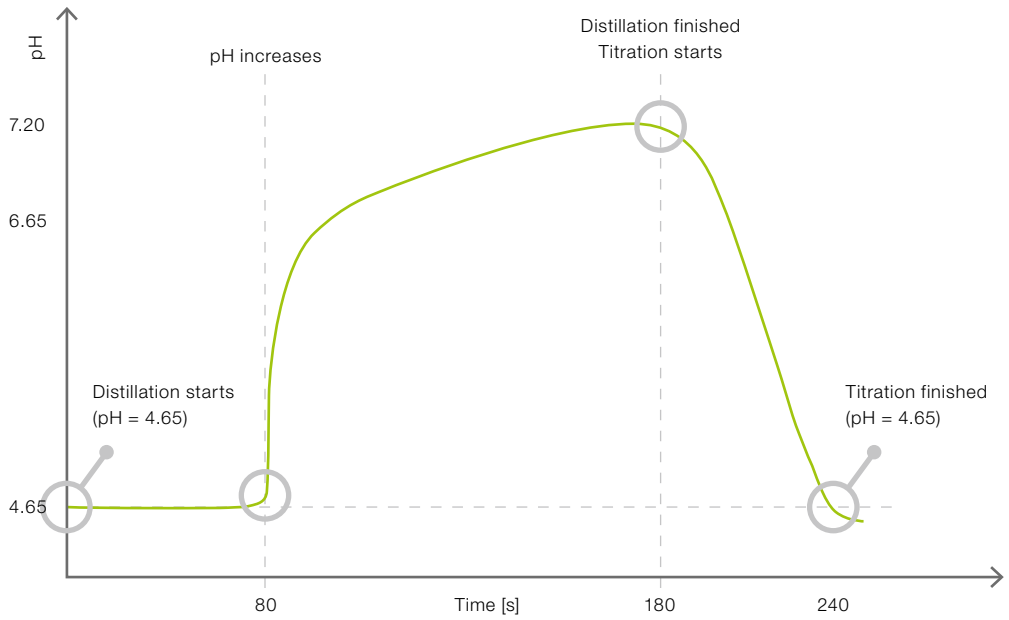


2.2 Optimizing distillation

The optimum distillation time can be determined as soon as the distillation unit has been conditioned and the time between distillations does not allow glassware to cool to the point where it affects the distillation volume. Conditioning (preheating) is performed by running a distillation sequence without a sample to bring the instrument up to operating temperature and cover the inner surface of the glass with a layer of hot water. The unit is considered conditioned once the temperature and moisture level can be kept constant from one distillation to the next. Reproducible results are then obtained.



The equipment may cool down between distillations if the time between runs is too long. That adversely affects the conditions for obtaining reproducible results. However, the right conditions can be restored by performing another distillation.

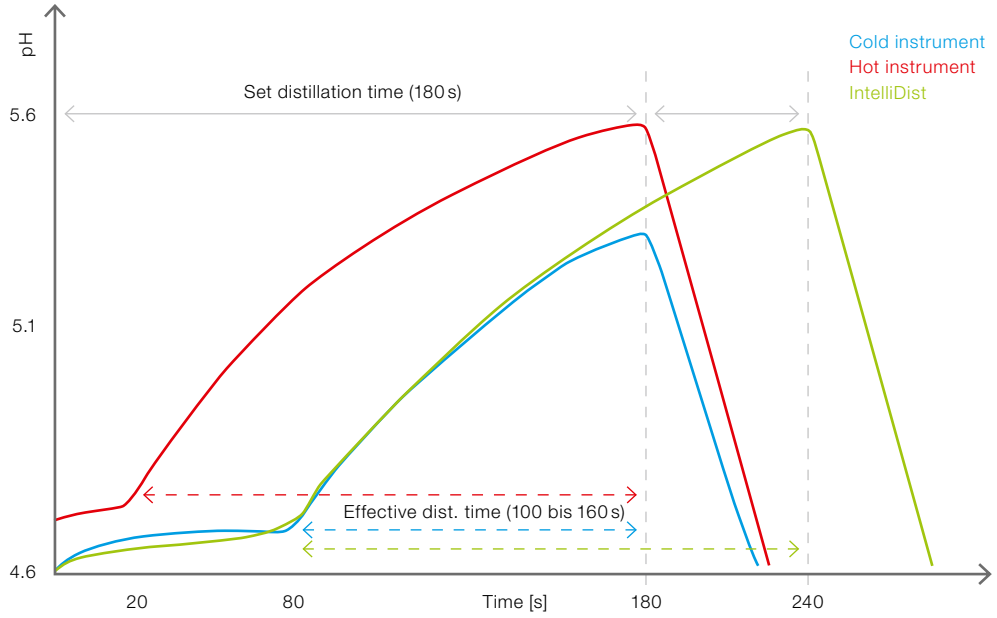


pH progression during distillation and titration with explanations

The graph shows the progression of the pH level in the boric acid receiving solution during distillation and titration. The initial pH level of the boric acid in the receiving vessel is 4.65. The time until the pH level in the receiving vessel starts to rise due to the distillate is variable and is related to the conditioning of the distillation unit. The time until the pH level first starts to rise can vary from 20 seconds for a conditioned unit to as much as 90 seconds for an unconditioned unit. For that reason, the reproducibility of the results is very closely linked to the conditioning of the instrument and sufficient distillation time for the samples.

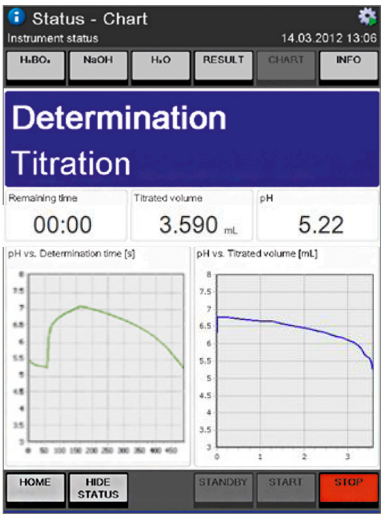
Reaching operating temperature

The intelligent and patented “IntelliDist” distillation mode on the KjelMaster K-375 makes it possible to achieve reproducible distillation without first conditioning of the distillation unit. It automatically detects when operating temperature has been reached and so reduces the determination time because preparatory stages such as preheating are no longer required.



Comparison of fixed distillation time and automatic IntelliDist function

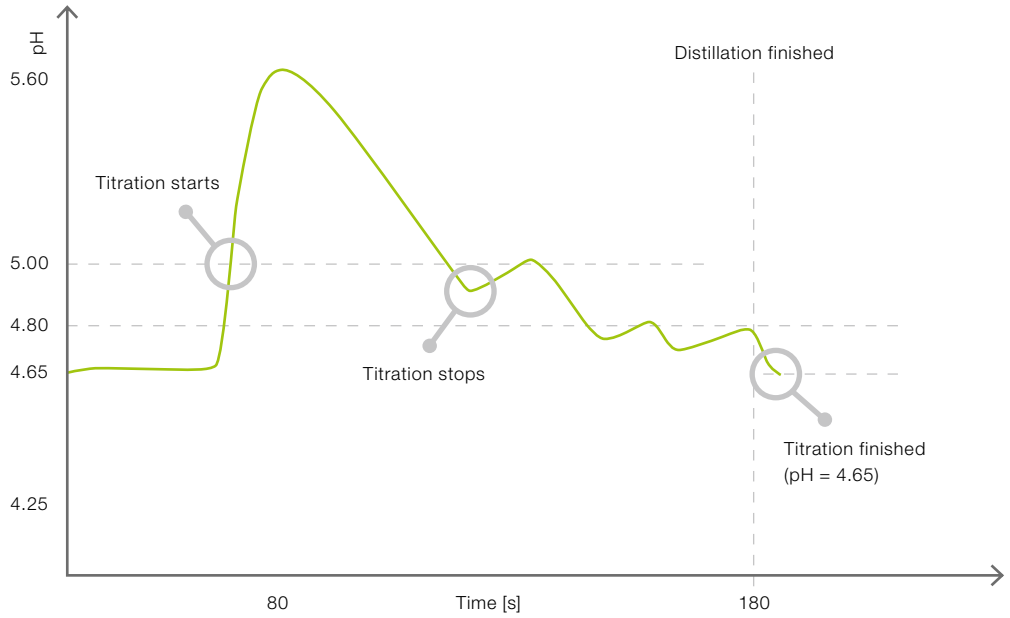
The optimum distillation time should be such that the nitrogen recovery of a reference substance such as ammonium dihydrogen phosphate is ≥ 99.5 %. Typical distillation times are between 150 and 300 s depending on the distillation unit used. With the KjelMaster K-375 you can monitor the distillation process on a graphical display.



KjelMaster K-375 screen showing graph of pH over time (left) and pH versus titrated volume (right)

Synchronizing distillation and titration

The overall determination time can be reduced by using the online titration mode. In the online titration mode, titration starts while distillation is still in progress as soon as a minimum threshold level is reached, as shown on the graph below. If the pH level is getting close to the desired final level before distillation has finished, titration is stopped and the pH level rises again. Titration is not continued to the final point until distillation has finished. Therefore, the synchronization of the two processes can save time without sacrificing accuracy.



Operating principle of “online titration”

2.3 Simplicity of decomposition versus recovery rate

Nitrogen in its oxidized nitrate and nitrite forms, and frequently nitrogen in aromatic heterocyclics as well, cannot be quantitatively determined using the Kjeldahl method because no ammonia is formed during the reaction with sodium hydroxide. The table below summarizes some of the most important resistant compounds for Kjeldahl nitrogen determination. It details a classification of compounds with function groups that contain organic nitrogen.

Resistant compounds that display a minimal contribution to Kjeldahl nitrogen

Connection	Recovery [%]
Nitrate, nitrite (organic and inorganic)	< 1
Aromatic N-heterocyclics: pyridine, pyrimidine, thiazole, imidazole, pyrazole	1 – < 80
Azo compounds	30 – 85
Hydrazine	< 50

A general chemical equation for describing oxidation has been published by Jurecek et al. [6][7] in the course of investigations into the oxidation of substances that contain organic nitrogen. Kjeldahl digestion of chemicals containing nitrogen can produce ammonia, nitrate or elemental nitrogen. A theoretical consideration of the chemical reaction in the Kjeldahl digestion has been published by Morita[8]. A concise form of the chemical equation[9] is shown below with a key to the indices a, b and c.

$$CHON_{a+b+c} + O \rightarrow a\ NH_3 + b\ HNO_3 + c/2\ N_2$$

a = Groups produce NH_3
b = Groups produce HNO_3
c = Groups produce N_2

The table lists organic functional groups correlated against the relevant nitrogen decomposition products a, b and c as described in the equation[10] above.

[6] M. Jurecek, Einige analytische Aspekte der Oxydation organischer Stickstoffverbindungen mit Chromsäure, Mikrochim. Acta 926 – 938, 5 (1962)
[7] V. Novak, P. Kozak, P. Matousek und M. Jurecek, Analytische Aspekte der Oxydation organischer Stickstoffverbindungen mit Chromsäure. Bestimmung der Nitro- und Nitrosogruppen, Mikrochim. Acta 1101 – 1107, 6 (1962)
[8] Y. Morita, A Theoretical Consideration on Chemical Reactions in the Kjeldahl Digestion, Bulletin of the Chemical Society of Japan, Vol. 41, 2029 (1968), A theoretical consideration on chemical reactions in the Kjeldahl digestion.
[9] P. Kozak, Vl. Novak, Zd. Bohackova und M. Jurecek, Analytische Aspekte der Oxydation organischer Stickstoffverbindungen mit Chromsäure, Oxydation aromatischer Azoverbindungen und Bestimmung der Azogruppen, Mikrochim. Acta 643 – 654, 4 (1963)
[10] Die Stickstoffbestimmung nach Kjeldahl, Die Umrechnung von Stickstoff zu Protein, Literaturstudie und Erfahrungsbericht, M. Ugrinovits, Büchi Laboratoriumstechnik GmbH, Göppingen

Correlation of organic function groups with relevant nitrogen decomposition products

Name	Function group	Decomposing product	Symbol
Amide	$-CONH_2$	NH_3	a
Amino	$-NH_2$		
Heterogeneous nitrogen	=N-		
Imino	=NH		
Isocyanide	-NC		
Iso-oxocyanate	-NCO		
Isothiocyanate	-NCS		
Oxocyanate	-OCN		
Peptide	-CONH-		
Nitrile group	-CN		
Hydroxyamine	-NHOH	HNO_3	b
Isonitro	-NOOH		
Nitro	$-NO_2$		
Oxim group	=NOH		
Nitroso	-NO	N_2	c
Azo	-N=N-		
Azino	=N-N=		
Diazonium	-N≡N+		
Hydrazone	-N-NH-R		
Hydrazine group	$-NHNH_2$		

The chemical decomposition paths encompass simultaneous reduction, dehydration, hydrolysis, substitution and other reactions. For $-NH_2$, =NH, ≡N und $[R_4N]_x$, the first step is protonation by sulphuric acid. The more alkaline an amine is, the easier it is to split the C-N bond. Primary amines are the most easily digestible amines.

Simplicity of reaction for a series of amines expressed as relative recovery rate compared to methylamine

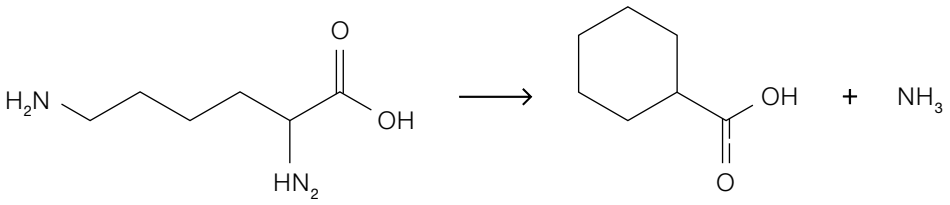
Type of amine	Simplicity of reaction[11]
Methylamine	100
Primary amines	90
Aniline	85
Secondary amines	80
Tertiary amines	76
Quaternary ammonium	54
2-nitroaniline	50

The next table compares the decomposition of a selection of typical amino acids. In the case of amino acids, the adjacent carboxyl groups weaken the C-N bonds and conversion to ammonia is simplified in comparison with primary amines. The dissolution of a C-N bond becomes more difficult if two amino groups are present, as illustrated by the example of lysine, because a stable piperidine carbonic acid is produced[12]. In that case, only around 50 % of the nitrogen is recovered. Amino acids that contain aromatic heterocyclics show lower nitrogen recovery rates; a rate of only 67 % is reported for tryptophan, for example. With modern optimized digestion equipment and methods, such as using selenium and mercury-free Kjeldahl tablets, higher recoveries can be achieved for tryptophan and lysine but the problem of more difficult digestion remains. In studies that are concerned with protein determination, an average nitrogen recovery rate of 98 % can be achieved[13].

Simplicity of decomposition according to type of amino acid

Type of amino acid	Simplicity of decomposition[14] [%]
Aspartic acid	100
Proline	98
Arginine	98
Tryptophan	67
Histidine	66
Lysine	50

[11] 100 % is easiest degradation, results based on nitrogen recoveries of Kjeldahl reactions without catalyst. S.P.L. Sörensen und A.C. Andersen (1905)
[12] Lässt sich der Stickstoffgehalt in Lysin und ähnlichen Verbindungen nach Kjeldahl bestimmen?, Hoppe-Seylers Zeitschrift für physiologische Chemie 44(5/6): 429–447
[13] Die Stickstoffbestimmung nach Kjeldahl, Die Umrechnung von Stickstoff zu Protein, Literaturstudie und Erfahrungsbericht, M. Ugrinovits, Büchi Laboratoriumstechnik GmbH, Göppingen
[14] 100 % is easiest degradation, results based on nitrogen recoveries of Kjeldahl reactions without catalyst, S.P.L. Sören und A.C Andersen (1905)



Structure of the amino acid lysine, which contains two amino groups and forms a stable piperidine carbonic acid

The length of a particular digestion process must be adjusted according to the simplicity of decomposition in order to obtain the highest possible recovery rate. Ammonium salts do not require a digestion step.

The next table shows the simplicity of decomposition as a function of the nitrogen-containing chemical group[15]. As revealed by the table, heterocyclics with a particularly high nitrogen content which demonstrate marked stabilisation by resonance of the aromatic system cannot be decomposed easily, or even at all in some cases. Organic nitrates and nitrites cannot be captured by Kjeldahl determination because the high oxidation level does not permit the formation of ammonia.

Groups containing nitrogen and typical recovery rates for nitrogen by Kjeldahl digestion

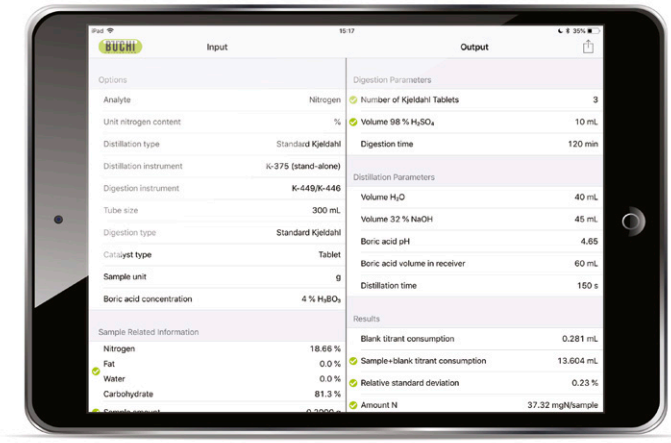
Group	N recovery by Kjeldahl
Azides (RN_3)	recovery rate approx. 20 %[a]
Azo compounds (-N=N-)	Only in some cases[b]
Carbamine group	Very good
Heterocyclics	The higher the resonance stability the lower the recovery
Hydrazine (NH_2-NH_2)	30 – 54 %
Imides, oximes	up to 100 %
Nitrates	1 %
Nitrides	10 %
Nitrites (Me-NO_2)	0 %
Nitro (R-NO_2)	50 %
Purines (uric acid, guanine, caffeine)	100 %
Acid amides	100 %

[a] Produces explosive HN_3
[b] Produces nitrogen N_2

[15] Die Stickstoffbestimmung nach Kjeldahl, Die Umrechnung von Stickstoff zu Protein, Literaturstudie und Erfahrungsbericht, M. Ugrinovits, Büchi Laboratoriumstechnik GmbH, Göppingen

2.4 Limit of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) are important features of analytical methods. The effect of boric acid concentration, the addition of potassium chloride and the concentration of the titration solution on the limits of detection and quantification were examined in the information bulletin[16] 'best@buchi 58/2010'. The quantification limit for nitrogen determination using the Kjeldahl methods is 0.02 mgN/sample, which defines the minimum sample size and titrant concentration. The optimum parameters for digestion, distillation and titration can be calculated with the help of the BUCHI Kjeldahl Optimizer[17] app.



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best@buchi No. 58
How to Achieve Low Detection and Quantification Limits for the Nitrogen Determination with Kjeldahl. www.buchi.com/media-center

[16] best@buchi Information Bulletin No. 58/2010, How to Achieve Low Detection and Quantification Limits for the Nitrogen Determination with Kjeldahl
[17] BUCHI Kjeldahl Optimizer, available for: Windows, iOS, Android and Windows Phone

The criteria for assessing the detection limit and quantification limit in Kjeldahl nitrogen determination are defined in the standard DIN EN 32 645. A rough estimate of the limit of detection (x_{LOD}) and the limit of quantification (x_{LOQ}) can be expressed by means of the following two equations:

$$x_{LOD} = \frac{3 \cdot s_B}{b}$$

$$x_{LOQ} = 3 \cdot x_{LOD}$$

s_B = Standard deviation of blank determination
b = Gradient of calibration curve

Ten blank values and a total of ten data points of equidistant calibration steps in the range x_{LOD} and x_{LOQ} are required as the basis for the analysis. s_B is established from the blank determination. The calibration curve consists of known quantities in standard solutions that are set against the experimental results. After determination of the limits of the analytical procedure, the results are reported according to a convention detailed in the next table.

Reporting of analytical results [DIN EN 32645]

Results	Expression	Additional information
$x \geq x_{LOQ}$	Content determined	Standard deviation
$x_{LOD} < x < x_{LOQ}$	Identified	Not identifiable, x_{LOQ}
$x < x_{LOD}$	Not identified	No higher than $2 \cdot x_{LOD}$

With the aid of titration, nitrogen quantities of as little as 0.05 mg/sample can be detected, albeit with a relative standard deviation of > 1 %. Based on analyses according to DIN EN 32 645 for the BUCHI distillation units, details of detection limits between 0.02 mgN and 0.2 mgN per sample can be found in BUCHI application notes. It can be assumed that, depending on the type of titration and the solution concentration, nitrogen quantities of 0.3–0.6 mgN per sample are sufficient for quantification.

At this point, a short discussion of the quantification limit and its practical effect on the optimization of sample sizes is appropriate. Animal feed and food samples such as milk, meat, cereal grain and beans contain high quantities of proteins, and so the nitrogen content to be determined is considerably higher than the determination limit. In that regard, fats and carbohydrates are the critical constituents of the sample as they have a tendency to foam and, therefore, require a large amount of sulphuric acid for decomposition.

Furthermore, samples that are rich in fat and carbohydrate usually have a low nitrogen content so that larger sample sizes are necessary in order to perform the x_{LOD} determinations referred to above. With samples that have a low N content there are limitations with regard to the sample size because of the size of the sample tube, the risk of foaming and the consumption of sulphuric acid and its ratio to the catalyst. In general a sample size of 5 g is the maximum for 300 mL sample tubes, whereas larger samples sizes can be used for Kjeldahl determination in 500 mL sample tubes. Larger sample sizes can be considered for Kjeldahl determination but attention should also be paid to optimizing

the process. That includes the use of anti-foaming agents, using hydrogen peroxide or programming temperature gradients for the digestion process.

For determining TKN (total Kjeldahl nitrogen), water-based samples are generally used, most of which evaporates during the digestion process. For such determinations, the detection and determination limits are often expressed as concentration levels [mg/L][18]. For TKN determination consisting of digestion and distillation phases, the 300 mL sample tubes can be used for samples up to 200 mL and the 500 mL tubes for samples up to 400 mL[19] [20]. Consequently, the nitrogen determination limits specified in [mg/L], sometimes stated in simplified terms as [ppm], are between 0.5 and 1 ppm if 300 mL samples tubes are used and between 0.3 and 0.6 ppm for 500 mL sample tubes.

Application Note Nr. 118/2013
Determination of TKN (Total Kjeldahl Nitrogen) in Water and Waste Water according to the Kjeldahl Method

Application Note Nr. 049/2010
Determination of Total Kjeldahl Nitrogen (TKN) in Water and Waste Water using 500 mL Sample Tubes)

Application Note Nr. 040/2010
Determination of Total Kjeldahl Nitrogen (TKN) in Water and Waste Water using 300 mL Sample Tubes

[18] BUCHI Application Note No. 118/2013, Determination of TKN (Total Kjeldahl Nitrogen) in Water and Waste Water according to the Kjeldahl Method
[19] BUCHI Application Note No. 049/2010, Determination of Total Kjeldahl Nitrogen (TKN) in Water and Waste Water using 500 mL Sample Tubes
[20] BUCHI Application Note No. 040/2010, Determination of Total Kjeldahl Nitrogen (TKN) in Water and Waste Water using 300 mL Sample Tubes

3. Nitrogen types

according to Kjeldahl and related determinations

1. TN
2. TKN
3. NH_3-N
4. TON
5. TON & TIN

In the chemical process of Kjeldahl digestion, organically bound nitrogen is oxidized to ammonia or ammonium ions. In the method according to Kjeldahl, the nitrogen is separated out in the form of ammonia by steam distillation and its quantity determined by titration.

Ammonia and ammonium ions, which are already present in the sample, are quantified collectively as TKN in addition to the organic nitrogen.

In routine analyses, inorganic nitrogen can also occur as well as Kjeldahl nitrogen. It may originate from nitrate or nitrite ions, for example. There are various chemical reactions which can convert inorganic nitrogen into ammonia or ammonium ions and make it accessible to determination methods according to Kjeldahl. A summary of the nitrogen terminology and definitions used in the literature[21] is presented in the table below.

Summary of nomenclature and definitions of nitrogen groups

Ammonia nitrogen	
Definition	Total of ammonium N and ammonia N in the acid-base pair NH_4^+/NH_3
Acronyms / Symbols	NH_3-N and/or NH_4^+-N

Kjeldahl nitrogen = Total Kjeldahl nitrogen	
Definition	Total of organic N and ammonia N
Acronyms / Symbols	NKjel, TKN

Organic nitrogen = Total Organic nitrogen = Dissolved organic nitrogen	
Definition	Difference expressed by TKN – Ammonia N
Acronyms / Symbols	Norg, TON, DON

Total oxidized nitrogen	
Definition	Total of nitrate N and nitrite N
Acronyms / Symbols	TON (not to be confused with total organic nitrogen)

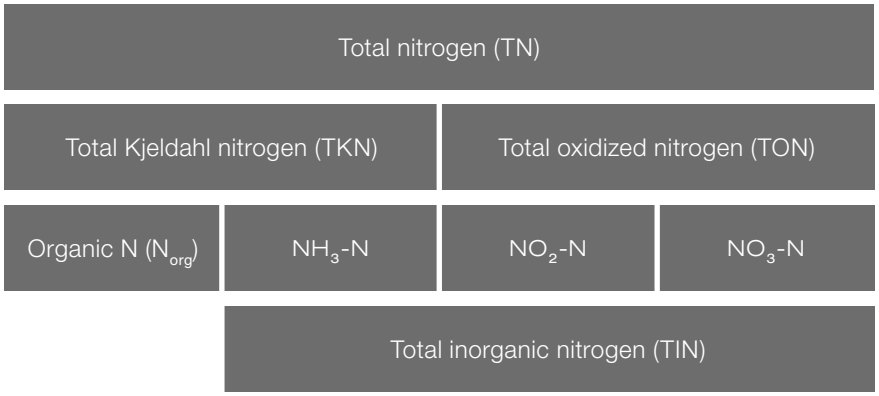
Total inorganic nitrogen = Dissolved inorganic nitrogen	
Definition	Total of ammonia N, nitrate N and nitrite N
Acronyms / Symbols	TIN, DIN

[21] W.M. Muirhead and K. Chapman, Water Environment & Technology, p 58–59, Vol 21, No. 4 (2009)

Total nitrogen	
Definition	Total of ammonia N, nitrate N, nitrite N and organic N
Acronyms / Symbols	TN

The table below summarizes the various types of nitrogen determination method which can be performed by means of digestion and/or steam distillation.

Different types of nitrogen determination method



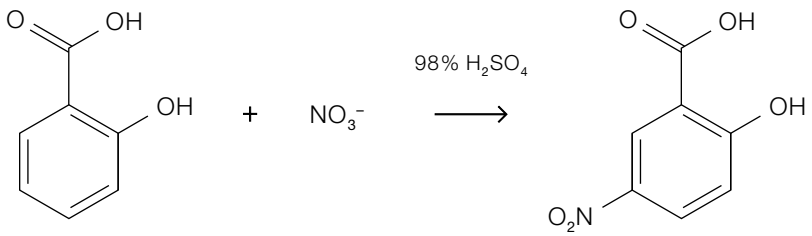
The term “Kjeldahl determination” and associated determinations was coined by authors in a variety of different areas of interest such as environmental analysis, water analysis and the food industry. A detailed explanation of nitrogen determination according to Kjeldahl and the associated equipment for digestion, distillation and titration is discussed in the book The Kjeldahl Knowledge Base. This chapter provides a more detailed explanation of the practical implementation of specific methods for direct determination of total nitrogen (TN) and ammonia nitrogen (NH_3-N). TN is the combined total of total Kjeldahl nitrogen (TKN) and total oxidized nitrogen (TON). Direct determination of ammonia nitrogen enables calculation of the organic nitrogen (N_{org}) and total inorganic nitrogen (TIN). The latter can be directly determined experimentally in inorganic samples by reduction with Devarda's alloy. The sections that follow examine the individual nitrogen classes in detail.

Application Note Nr. 235/2016
Ammonium, nitrate, and total nitrogen determination in fly ash

3.1 TN (Total nitrogen) determination

The determination of total nitrogen involves establishing the sum total of TKN and TON. The official AOAC Method 955.04 D describes the determination of the total content of nitrogen in samples that contain nitrate[22] [23]. In the chemical reaction, salicylic acid is nitrated in an electrophilic aromatic substitution with the help of sulphuric acid, resulting in 5-nitrosalicylic acid.

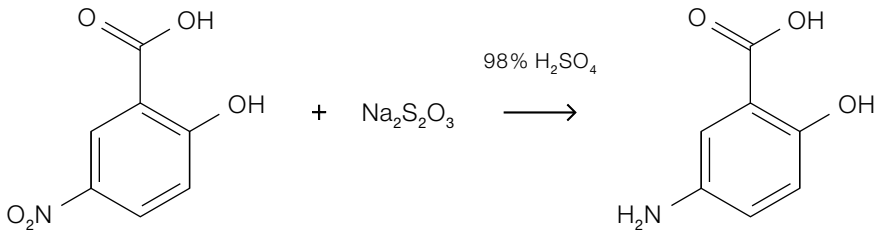
1. Electrophilic aromatic substitutions of salicylic acid



Salicylic acid | Nitrate | 5-nitrosalicylic acid

In the second stage of the chemical reaction, 5-nitrosalicylic acid is reduced to 5-aminosalicylic acid by sodium thiosulphate.

2. Reduction of nitrosalicylic acid

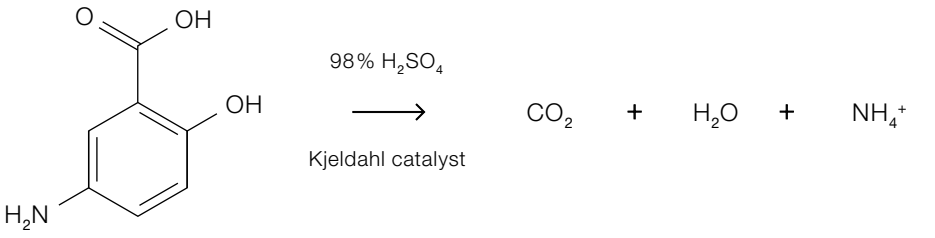


5-nitrosalicylic acid | Sodium thiosulphate | 5-aminosalicylic acid

5-amino salicylic acid can be captured with the Kjeldahl method; the results are determined by titration and the TN (total nitrogen) calculated. TN equates to the sum total of TKN and nitrate/nitrite nitrogen as shown in the above table.

[22] AOAC Official Method 955.04 (2.4.03), 1998
[23] BUCHI Application Note 2016/252 Selective nitrogen determination methods related to Kjeldahl

3. Kjeldahl nitrogen determination



TN determination in fertilizer

Processes for determining TN in fertilizers are described in the AOAC, ISO and EN standards. The basic concept is to reduce the TON (total oxidized nitrogen) in a first stage of the process and then to establish the TKN in a Kjeldahl nitrogen determination process[24 25 26]. The AOAC method involves reduction with salicylic acid, the ISO method describes reduction by means of chrome powder in an acidic medium and EN 15750 B specifies reduction of nitrate using iron powder and tin (II)-chloride in an acidic medium. All of the methods described have limitations regarding their applicability when dealing with specific problem situations. The AOAC method is restricted to liquid samples and limited in the presence of a high Cl : NO_3 ratio. However, liquid fertilizer sample weights of up to 1 g can be analysed with a good degree of reproducibility. Higher sample weights, on the other hand, would result in lower recovery rates of nitrogen from nitrate[27]. The use of mercury oxide (HgO contained in the catalyst mixture) is specified for this method and detoxification of mercury by means of sulphide and thiosulphate solution suggested. The use of thiosulphate in acidic media leads to the formation of elementary sulphur, which is sublimated into the glassware, resulting in contamination of the glassware. That effect can be avoided by using zinc dust as the reducing agent instead of thiosulphate. Mercury oxide can be simply and entirely replaced by more modern catalyst mixtures based on less toxic metallic salts, as described in the more recent ISO and EN publications. EN Method A and the ISO method are limited purely to nitrates and/or ≤ 60 mg of nitrate nitrogen.

Application Note Nr. 041/2010
Nitrogen Determination in Nitrate Containing Fertilizers according to AOAC 955.04-D (Kjeldahl Method)

Application Note Nr. 310/2018
Nitrogen determination in compound fertilizer according to Devarda

[24] AOAC Official Method 955.04, Nitrogen (Total) in Fertilizers, Kjeldahl Method.
[25] ISO 5315:1984, Fertilizers – Determination of Total Nitrogen content – Titrimetric method after distillation.
[26] EN 15750:2009, Fertilizers – Determination of Total Nitrogen in fertilizers containing nitrogen only as nitric, ammoniacal and urea nitrogen by two different methods.
[27] BUCHI Application Note AN 041/2010, Nitrogen Determination in Nitrate Containing Fertilizers according to AOAC 955.04-D (Kjeldahl Method).

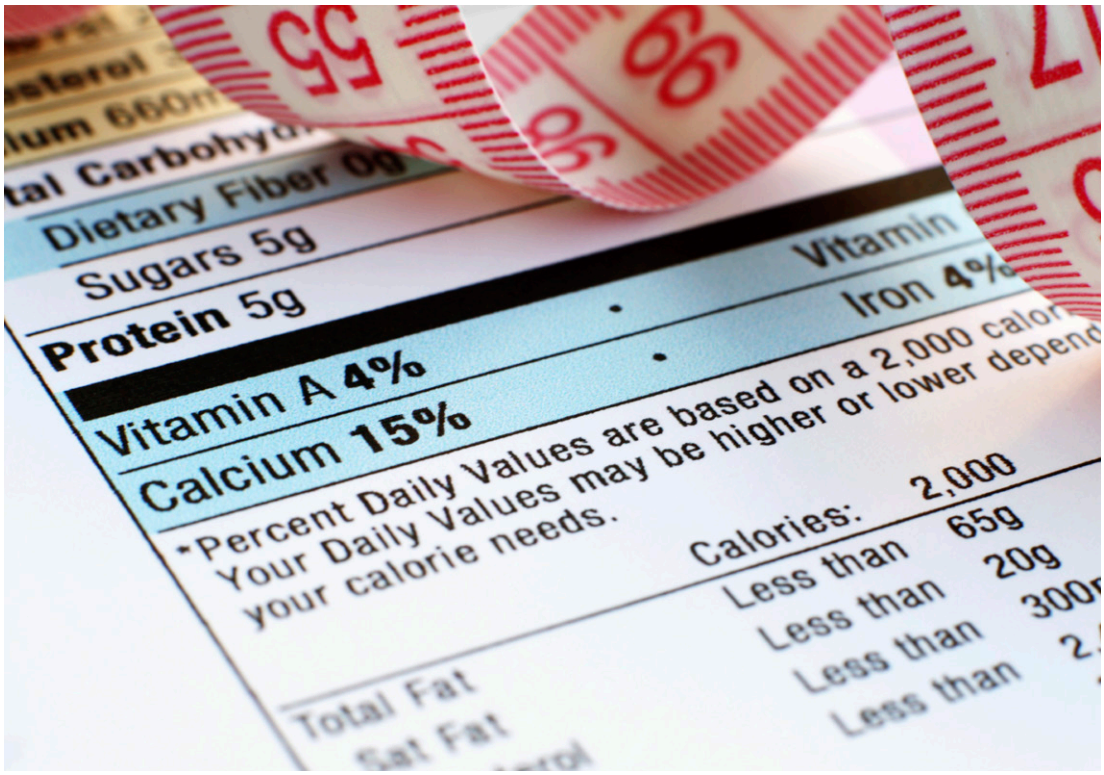
3.2 TKN (Total Kjeldahl nitrogen, N_{org})

Regular Kjeldahl nitrogen determinations encompass digestion, distillation and titration of the sample. Depending on the area of interest, either the term “Kjeldahl nitrogen” or “total Kjeldahl nitrogen” is used to describe the total of organic nitrogen and ammonia and ammonium nitrogen. Food samples do normally not contain any ammonium or ammonia and the determination of nitrogen produces organic nitrogen N_{org}, which can be linked to the protein content. That is done by multiplying the nitrogen content by an empirical protein factor (e.g. 6.25). In the area of environmental protection, especially the analysis of waste water, the term TKN is used if the organic nitrogen is determined together with the ammonia nitrogen according to the Kjeldahl method after the evaporation of water during the digestion process.

$TKN = N_{org} + NH_3\text{-}N$

TKN in environmental analysis

In environmental analyses (e.g. waste water) in which nitrogen determination is performed by means of the Kjeldahl method, liquid samples are used and the first stage of the digestion process primarily involves evaporation of water. In addition to organic nitrogen, waste water samples may also contain ammonia and ammonium nitrogen, which is included in the figure for the nitrogen content determined. This type of Kjeldahl determination is referred to as total Kjeldahl nitrogen (TKN) determination. TKN in water-based samples is typically associated with a nitrogen content in the ppm range.



N_{org} in food analysis

Food and animal-feed products consists mainly of fat, protein, fiber, carbohydrates, ash (total mineral content) and moisture (water). Because the protein content plays an important role not only in the price of raw materials (e.g. milk) but also the nutritional information declared on the product packaging. The product Nutrition Facts are subject to regular verification.

The protein content also serves as a key indicator for staple foods, has an effect on calorific content, is a quality parameter (e.g. milk, meat) and affects the production process. Proteins are integral in terms of their nutritional value for humans and animals and are contained in drinks, foods and animal feeds[28]. The nitrogen content of proteins is roughly 16 %, resulting in a general protein factor of 6.25. The protein content is calculated from the experimentally established nitrogen content multiplied by the specific protein factor.

Protein = N_{org} * Protein factor

3.3 Ammonia nitrogen determination (NH_3-N)

Ammonia (NH_3) and ammonium ions (NH_4^+) can be determined by direct water vapour distillation of slightly alkaline samples with a pH of 9–10. If only ammonia is to be quantitatively determined from a water-based solution using direct distillation, it can be done with a neutral or slightly basic pH level of > 7. In a neutral methanol solution that contains barium chloride, ammonia can be separated by direct distillation from a sample of a cosmetic product, for example[29]. Another procedure describes determination using a hydrogen phosphate buffer at a pH of 7 in which ammonia can be distilled directly from water and waster water samples. A borate buffer at a pH of 9.5 is recommended in the EPA method for direct distillation of ammonia nitrogen in drinking water, surface water and salt water, and in domestic and industrial waste[30]. If ammonium is to be determined, mildly alkaline conditions in a pH range of 6.0 to 7.4 can be created by the addition of magnesium oxide according to a standard ISO method. Such conditions are recommended for the determination of ammonium by distillation and titration in water analysis[31].

[28] Souci Fachmann Kraut, Food Composition and Nutrition Tables, 6th revised and completed edition (2000), compiled by Heimo Scherz und Friedrich Senser, medpharm Scientific Publishers, ISBN 3-88763-076-9

[29] Methods of analysis necessary for checking the composition of cosmetic products, Commission Directive 83/514/EEC

[30] ISO 5664-1984 – Water quality – Determination of ammonium – Distillation and titration method

[31] EPA Method 350.2 – Nitrogen-Ammonia, Distillation Procedure

3.4 Calculation of TON (Total organic nitrogen, N_{org}, DON)

The organic nitrogen cannot be determined directly and instead is calculated according to the following formula:

N_{org} = TKN – NH_3-N / NH_4-N

In normal Kjeldahl nitrogen determination in food and animal-feed samples, neither ammonia nor ammonium salts are expected, at least not in concentrations comparable with the organic nitrogen. For that reason, the simplified equation N_{org} = TKN applies in this case and provides N_{org} from a single experiment encompassing digestion, distillation and titration. In food and animal-feed analysis it is a recognised method of calculating the protein content by multiplication of the established TKN by a sample-specific empirical protein factor.

Determining the casein content of milk

Caseins are the chief proteins that make up nearly 80 % of the proteins in cow's milk. Caseins do not pass into the whey and are subsequently processed accordingly, e.g. in the production of cheese. An ISO specification describes the principle as follows[32]:

Casein is precipitated from a random sample of the milk by adding acetic acid and sodium acetate solution so that the final pH level of the mixture is around 4.6. The precipitated milk casein is separated out by filtration. The nitrogen content of the precipitation is determined according to the VDLUFA VI C30.4 method for milk[33].

Determination of NPN (non-protein nitrogen) in milk

In the first stage of NPN determination, the protein is precipitated using trichloroacetic acid as in casein determination. Afterwards, however, the filtrate is analysed according to the Kjeldahl method. The ISO 8968-4 specification describes the principle as follows:

The protein is precipitated from a random sample by the addition of trichloroacetic acid so that the final concentration of trichloroacetic acid in the mixture is roughly 12 %. The precipitated milk protein is removed by filtration and the remaining filtrate contains the non-protein constituents.

The nitrogen content of the filtrate is determined according to the ISO 8968 method for milk. The non-protein nitrogen (NPN) fraction consists of urea and other low-molecular-weight compounds containing nitrogen, such as creatine and creatinine. Approximately 50 % of the NPN in milk is urea and the variation of the NPN is attributable chiefly to the urea variation. NPN has a low nutritional value and does not contribute to the cheese yield.

Application Note Nr. 050/2010
Non Protein Nitrogen Determination in Milk according to the Kjeldahl Method

Application Note Nr. 051/2010
Casein Determination in Milk according to the Kjeldahl Method

[32] ISO 17997-2:2004, Milk – Determination of casein-nitrogen content, Part 2: Direct method
[33] BUCHI Application Note No. 051/2010 Casein Determination in Milk according to the Kjeldahl Method

3.5 Determination of TON (Total oxidized nitrogen) and TIN (Total inorganic nitrogen)

The Kjeldahl digestion process can be used for the determination of organically bound nitrogen but would not produce any results with samples containing nitrates and nitrites. For determining the nitrogen in nitrates and nitrites, Devarda's alloy is used to reduce it to ammonia, thereby making it accessible to the Kjeldahl method.

As well as using Devarda's alloy, other reducing distillations can be performed using Fe(II) or salicylic acid as the reducing agent[34].

Those methods are suitable for the determination of nitrate and nitrite together with the ammonia nitrogen ultimately also present in the sample. A typical application is the determination of nitrogen in saltpeter (potassium nitrate). The process cannot be used in the presence of organic compounds, calcium cyanamide or urea. The chemical reaction consists of the reduction of the oxidized nitrogen compounds nitrate and nitrite to ammonia. Devarda's alloy reacts with sodium hydroxide to produce hydrogen, thereby reducing nitrate and nitrite to ammonia, which is captured in boric acid solution or sulphuric acid and titrated with sulphuric acid or sodium hydroxide. Typical parameters are described in the table below and the BUCHI application note[35].

A reducing distillation process for determining TON (total oxidized nitrogen) is carried out on a solid inorganic sample that contains nitrate and nitrite but no ammonium salts. In the determination of TIN (total inorganic nitrogen), by contrast, a sample containing not only nitrite and nitrate but also ammonium salts are analysed.

Typical parameters for reductive distillation for the determination of TON or TIN in a solid inorganic sample that contains nitrate, nitrite and ammonium salts or nitrate, nitrite and no ammonium salts.

Parameter	Value	Unit	Note
Reaction time	5	min	Reduction time
Water	0	mL	
NaOH	20	mL	
Distillation time	300	s	
Sample tube type	300	mL	Standard size
Titration type	Boric acid titration		
Boric acid	4 %	g / 100 g	
Titration solution	0.5	N	
Titration type	End point		
End point	4.65	pH	Inflection point

[34] Nitrogen-Information No. 7, Determination of nitrogen content in fertilizers containing nitrate: Iron reduction method, Devarda method, Salicylic acid method, BUCHI Application Report (1994)

[35] BUCHI Application Note No. 2016 / 252 – Selective nitrogen determination methods related to Kjeldahl

TIN (total inorganic nitrogen)

In environmental analyses (e.g. waste water applications) in which nitrogen determination is performed by means of the Kjeldahl digestion method, liquid samples are used and in the first stage of the process the water in the sample is evaporated. As well as organic nitrogen, waste water samples may also contain ammonia and ammonium nitrogen, which will be included in the figure for the nitrogen content determined. This type of Kjeldahl determination is referred to as total Kjeldahl nitrogen (TKN) determination. TKN in water-based samples is typically associated with a nitrogen content in the ppm range.

In the presence of organic substances, TIN cannot be directly determined by analysis but rather is calculated as follows:

$$TIN = TN - TKN + NH_3\text{-}N$$

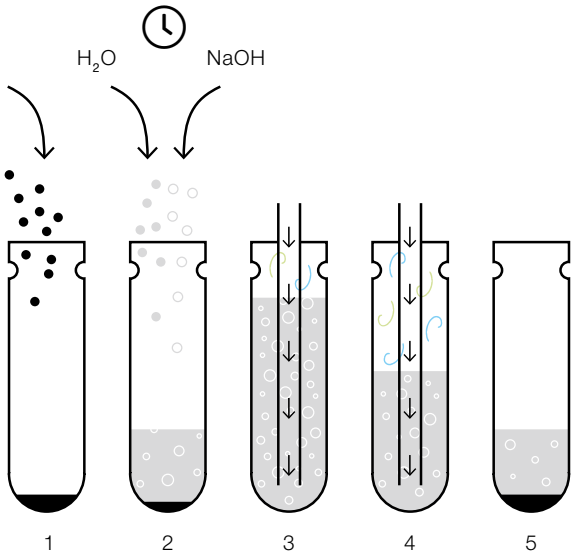
In the case of inorganic samples, TIN can be determined by reduction of nitrate and nitrite using Devarda's alloy.

At the end of the 19th century, Arturo Devarda, an Italian chemist, published a method which enabled the chemical reduction of nitrate and nitrite nitrogen under alkaline conditions using an alloy of aluminium (44–46 %), copper (49–51 %) and zinc (4–6 %)[36]. The method was primarily used for analysing nitrate in Chile saltpeter (sodium nitrate)[37][38]. The reduction of nitrate by Devarda's alloy is described by the following equation:

$$3\ NO_3^- + 8\ Al + 5\ OH^- + 18\ H_2O \rightarrow 3\ NH_3 + 8\ [Al(OH)_4]^-$$

The process consists of the reaction of an alkaline sample with Devarda's alloy, followed by steam distillation to separate the ammonia formed during the reduction process, and then determination of the ammonia by titration. More information on the method can be found in the BUCHI application notes.

[36] Devarda, A., Fields, J. (1899), Über Stickstoffbestimmung, Zeitschrift der analytischen Chemie 38 (1), 55 – 57.
[37] Devarda, A. (1892), Über die direkte Bestimmung des Stickstoffs im Salpeter, Chemiker Zeitung 16:1952
[38] Devarda, A (1894), Eine neue Methode zur Bestimmung des Stickstoffs im Chilesalpeter, Analytical and Bioanalytical Chemistry 33 (1): 113 – 114



1. Devarda reagent is added to the sample tube.
2. H_2O and NaOH are added, the reaction time is started. The alkalization reduces the Devarda reagent and H_2 is formed. The resulting H_2 in turn reduces nitrite and nitrate to ammonia.
3. Start of distillation: Strong foam formation becomes visible if H_2 is not completely consumed by the sample.
4. During distillation: the intensity of foam formation decreases over time
5. After distillation: still small H_2 gas bubbles visible

The Devarda reaction

To optimize the Devarda application, BUCHI offers a special glass splash protector to prevent carry-over. Due to the powerful reaction caused by Devarda's alloy, a sodium hydroxide-hydrogen aerosol is created which can be trapped by the additional glass chicane.

Devarda splash protector with additional chicane for preventing carry-over

Application Note Nr. 310/2018
Nitrogen determination in compound fertilizer according to Devarda

TON (Total oxidized nitrogen)

Oxidized nitrogen refers to the total inorganic nitrate and nitrite. It cannot be determined by reduction of nitrate and nitrite with the help of steam distillation because the distillation would result in determination of the total inorganic nitrogen (TIN). There are spectral photometry methods for TON determination which are used in water analysis[39]. They involve the reduction of nitrate to nitrite by hydrazine under alkaline conditions. The total nitrite ions then react with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride under acidic conditions to form a pink azo colourant. The absorption is measured at 540 nm and relates to the TON concentration determined by means of a calibration curve. However, the TON can be calculated by applying the following equation from the experimental TIN result and the ammonia N content.

$TON = TIN - NH_3\text{-}N$

[39] EPA Method 353.1


BUCHI
pH
9.21
HAMILTON

4. Qualification, verification and fault identification

1. Digestion
2. Distillation
3. Reference substances
4. Fault identification

For laboratories in the pharmaceutical field and those that work under GMP or similar requirements, it is imperative to be able to verify that the equipment used is functioning correctly. IQ (installation qualification), OQ (operational qualification) and PQ (performance qualification) are procedures aimed at validating equipment functional capability.

IQ/OQ certification plays a central role in ensuring a specific degree of measurement accuracy and reproducibility which can be achieved and documented. To that end, BUCHI offers complete IQ/OQ sets that can be performed by BUCHI-qualified staff.

Distillation units, for example, are evaluated by running a determination process on a pure, dry ammonium salt of which the nitrogen content is known. The ratio of the determined nitrogen content to the expected content is expressed as the percentage recovery. Recovery rates in the range of 98–102 % are evidence of a well functioning process.

IQ/OQ labels

Qualification processes are an important part of an overall method validation process. Ultimately, according to the GMP regulations, the user is responsible for the qualification process.

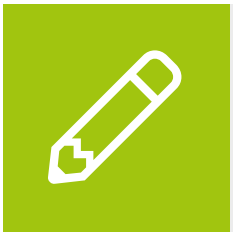
BUCHI offers IQ/OQ documentation including test equipment and execution. The qualification processes are based on the specifications and the knowledge gained about the equipment during the development process.

The available IQ/OQ test procedure offered by BUCHI covers a broad spectrum of device-specific system functionality.

Qualification is a prerequisite for good production practices and additional regulatory requirements such as are imposed by the US FDA (Food and Drug Administration), for example: “Create documented evidence that offers a high level of certainty that a certain process consistently produces a product that matches its defined specifications and quality features.”

Pharmaceutical companies are required to carry out an equipment qualification process. That is a formal process that provides documented evidence that a piece of equipment is suitable for its intended purpose and is maintained in a regularly serviced and calibrated condition that conforms to its use.

Equipment qualification is subdivided into four stages:



Design qualification (DQ)
Defines functional and operational specifications of the equipment.

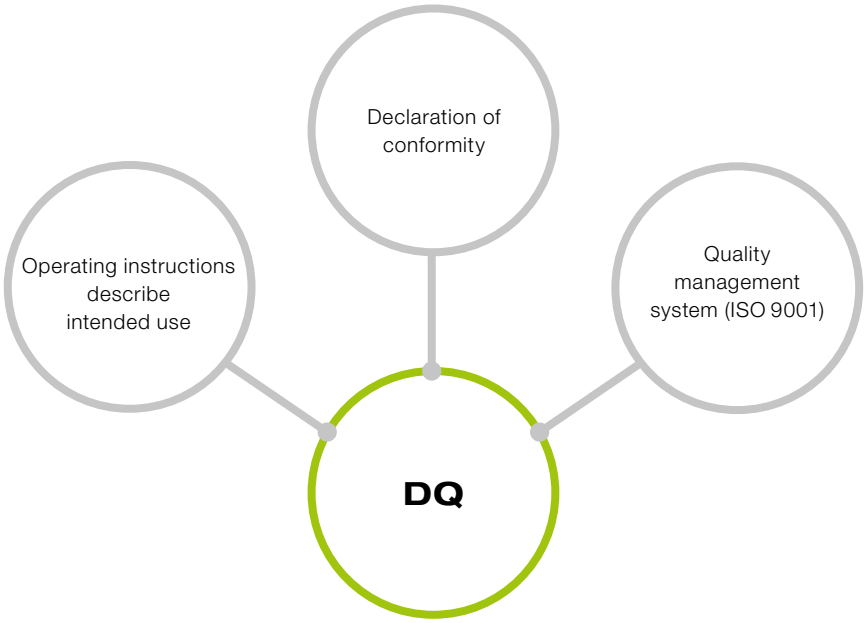
Installation qualification (IQ)
Ensures that the equipment has been received as designed and specified, that it is installed correctly in the chosen environment and that the environment is suitable for operation of the equipment.

Operational qualification (OQ)
Verifies that the equipment will function in accordance with its operating requirements in the selected environment.

Performance qualification (PQ)
Documents that the equipment works consistently according to the specifications and is suitable for its routine use.

Design qualification (DQ)

As part of the DQ process, the supplier should be qualified. How is that done? The roles of the individual stages are summarized in the chart below. Based on the predefined requirements from the customer's perspective and on legal requirements, a set of user requirement specifications (URS) can be produced and compared with the specifications of the equipment manufacturer.



Installation qualification (IQ)

IQ ensures that the equipment has been correctly received and installed in the selected environment. The work environment is documented and inspected to ensure that it is suitable for operation of the equipment.

IQ is the documented verification that systems and equipment as installed or modified match the approved design and recommendations of the manufacturer.

The BUCHI IQ process documents that the equipment has been delivered complete with all components and has been installed according to the BUCHI specifications.

It comprises the identification of all system components, software applications including operating system, safety and environmental aspects and the supply of the complete user documentation.

Operational qualification (OQ)

Equipment functions are checked to verify correct operation in accordance with the manufacturer's specifications.

The BUCHI OQ process documents that the equipment is functioning precisely according to the BUCHI specifications. Requirements regarding qualification of the general performance of the system are provided. The documents consist of precise written descriptions, expected results and tolerances. The OQ testing normally follows the IQ process.

In addition, periodical OQ testing can be incorporated in the ongoing qualification processes of a business to meet the regulatory requirements.

In the IQ/OQ documents BUCHI offers customers a documented and reliable means of monitoring the delivery, installation and performance of the Kjeldahl system. They show that the complete system functions as specified.

Performance qualification (PQ)

PQ is defined according to the customer’s application and is also carried out by the customer. That can be done regularly or periodically by the determination of reference substances. The expected results and tolerances can be adjusted as required in this case.

4.1 Qualification of a digester unit

In the course of OQ, the device and all necessary peripheral equipment, accessories and other components of the system are tested according to a specified method. OQ also ensures that the device and all other components match the specifications in the operating instructions.

BUCHI recommends that OQ is carried out once a year, though different time intervals can be agreed in individual cases.

OQ testing procedure:

- Acceptance of documentation by customer
- Verification that IQ has been correctly carried out and that the document is available and filed in the qualification folder.
- Verification that all measurement instruments, tools, equipment and reagents are available together with the relevant certificates where applicable
- Function testing
- Temperature testing
- Completion of a report summarising the completion of the entire OQ process
- Acceptance of the OQ by the customer

4.2 Qualification of a distillation unit

OQ testing procedure:

- Acceptance of documentation by customer
- Verification that IQ has been correctly carried out and that the document is available and filed in the qualification folder.
- Verification that all measurement instruments, tools, equipment and reagents are available together with the relevant certificates, etc. where applicable
- Function testing (including components and peripherals)
- Performance test using a reference substance for nitrogen determination
- Completion of a report summarising the completion of the OQ process
- Acceptance of the OQ by the customer

The performance test with the reference substance forms the key element of the OQ. If the reference substance contains moisture, it must be dried beforehand. That additional step is advisable if the resulting recovery rate of a preceding test failed to provide results in the range of 98–102 %. If drying of the reference substance is necessary, use 5–10 g of the reference substance and dry it in the drying oven at a temperature of 100–105 °C for 8 hours. Allow the salt to cool to room temperature in a desiccator filled with activated silica gel or other drying agents. Once it has cooled down, place the reference substance in a sealed glass jar.

If the titrator is not integrated in the unit, it should similarly be qualified according to the OQ procedure of the manufacturer concerned to eliminate any possible sources of errors.

4.3 Reference substances

Reference substances are substances with known nitrogen content which are used to verify the function of the system and the complete application. Regular analysis of reference substances is recommended.

Name	Purity [%]	% N theoretical (100 % purity)	Digestion necessary
Ammonium dihydrogen phosphate	99.5	12.18	No
Ammonium sulfate	99.5	21.21	No
Glycine	99.7	18.66	Yes
Phenylalanine	99.0	8.47	Yes
Tryptophan	99.0	13.72	Yes
Acetanilide	99.0	10.36	Yes

The distillation unit is tested without the digestion phase using a standardized ammonium salt (e.g. ammonium dihydrogen phosphate).

To test the entire Kjeldahl process including the digestion phase, standardized amino acids such as tryptophan[40] are used.

Calculation

The determination of reference substances is performed as for normal samples. First of all, the nitrogen content in percent is calculated. The following example relates to boric acid titration. Other calculations are shown in the *Kjeldahl Knowledge Base*.

$$\%(N) = \frac{(V_{sample} - V_{blank\ value}) \cdot M(N) \cdot c_{acid} \cdot f \cdot z}{m_{sample} \cdot 10}$$

%(N)	Uncorrected nitrogen percentage	
V_{sample}	Consumption of titrant for the sample	mL
$V_{blank\ value}$	Consumption of titrant for the blank value	mL
M(N)	Atomic mass of nitrogen	14.00674 g/mol
c_{acid}	Concentration of titrant	mol/L
m_{sample}	Sample weight	g
f	Titre	
z	Equivalence factor	

[40] ISO 8968-2:2001, Milk – Determination of nitrogen content – Part 2: Block-digestion method (Macro method)

Taking account of the purity of the reference substance for $\%N(_{det})$, the results are calculated as a percentage of nitrogen and the recovery rate.

$$\%(N)_{det} = \frac{\%(N) \cdot P}{100}$$

$$R = \frac{\%(N)_{det} \cdot 100\,\%}{\%(N)_{theor}}$$

$\%(N)_{det}$	Corrected nitrogen percentage for the purity of the sample
%(N)	Uncorrected nitrogen percentage
P	Purity [%]
R	Recovery rate [%]

Results

The relative standard deviation (RSD) of the blank values should be lower than 5 %. If the RSD is higher, blank value determination should be repeated until the condition is satisfied for three successive blank values. The recovery of the reference substance should be between 98 and 102 % and the RSD for $\%(N)_{det}$ should be less than 1 %.

Application Finder
Look up detailed information on a large number of sample matrices.
www.buchi.com/applications

KjeldahlOptimizer App
Optimize individual Kjeldahl methods.
www.buchi.com/kjeldahloptimizer

KjelReports App
Easily calculate results and produce reports for Kjeldahl and SO_2 determinations.
www.buchi.com/kjeldahlreports

4.4 Troubleshooting

Problems, causes and corrective measures

Problem	Cause	Corrective measure
Nitrogen content too high	Air in titration system/burette/tubing	Rinse and refill burette
	Carry-over during distillation	Use less volume or increase water volume for dilution
	Incorrect titrant	Use correct concentration, titration volume for samples should be 3–17 mL
	Calculation error	Check calculation, titration concentration, molar equivalence factor and titer
	Defective pH electrode	Calibrate electrode, replace if necessary
	Defective colorimetric sensor	Clean sensor and mesh fabric, replace if necessary
	Air bubbles interfering with the colorimetric titration	Check position of protection mesh and correct as required
	Dirty glassware	Only use clean glassware
Nitrogen content too low	Incomplete digestion	Increase digestion time
	Insufficient H_2SO_4 volume	Increase volume
	Incorrect ratio of Kjeldahl tablets to H_2SO_4	Correct ratio
	Nitrogen content per sample tube is too high	Do not weigh more than 200 mg of nitrogen per sample
	Insufficient NaOH or incorrect concentration of NaOH (32 % required)	Adjust the volume until colour change is visible
	Leakage during distillation	Check connection between condenser and splash protector, replace seal if necessary
	Leakage during digestion	Check seals and scrubber vacuum capacity
	Incorrect titrant used	Check and correct
	Defective pH electrode	Calibrate electrode, replace if necessary
	Defective colorimetric sensor	Clean sensor and mesh fabric, replace if necessary

Problem	Cause	Corrective measure
Poor repeatability	Air bubbles interfering with the colorimetric titration	Check and correct the position of the protection mesh
	Dirty glassware	Only use clean glassware
	Air bubbles in titration system/burettes/tubing	Repair tube and refill burette
	Aspiration not functioning properly	Check for leaks and repair as necessary
	pH electrode incorrectly calibrated/not calibrated	Calibrate electrode with fresh buffer
	Setpoint is outside of the specified range (only applies to colorimetric determination)	Repeat setpoint determination using freshly prepared boric acid; check mesh
	Sample not homogeneous	Improve homogenisation of sample
	Weighing problems	Use weighing boats to improve the process
	Incomplete digestion, digestion time too short	Check the sample colour during digestion and adjust the digestion time accordingly
	Vacuum capacity is too high during digestion	Reduce vacuum capacity on scrubber by means of the bypass valve
	Stirrer is not working	Clean or replace stirrer
	Immersion tube of autosampler blocked, loose, too short or defective	Check and correct
	Air bubbles interfering with the colorimetric titration	Check and correct the position of the protection mesh, increase water volume to make reaction milder
	Incorrectly positioned titration metering tip	Check and correct positioning
	Indicator ageing (only applies to colorimetric determination)	Replace boric acid and indicator using fresh solutions
	Incorrect ratio of indicator to boric acid or incompatible indicator	Check and correct according to BUCHI application notes
	Sensor cable loose connection	Check and correct

4.4.1 Typical mistakes in digestion

Crystallization of the sample after digestion

- Incorrect ratio of H_2SO_4 to catalyst
- Digestion time too long
- Vacuum capacity of scrubber too high
- Leakage in suction module
- Defective seals or glassware

Samples are not clear

- No catalyst used or too little catalyst used
- Digestion time too short
- Temperature too high → Sealant flushed into sample tube
- Digestion of samples containing phosphate, liquid remains turbid

Escape of digestion gases

- Seals are defective
- Vacuum capacity of scrubber too low
- Leakage in the system, e.g. tube connector leaking
- Blocked tubing
- Vacuum reduced on bypass valve
- Scrubber pump corroded or blocked

Delayed evaporation / foaming

- Boiling rods missing or boiling chips used
- Anti-foaming tablets or other anti-foaming agents missing
- Start temperature too high during digestion

4.4.2 Typical mistakes in distillation

Samples do not turn dark blue/brown after addition of NaOH

- NaOH canister empty
- Air in NaOH tube
- No catalyst (only H_2O_2) or a catalyst with a very low copper content used for digestion

Pronounced splashing when adding chemicals or during distillation

- Incorrect sample tube size chosen
- Volume in sample tube is too high
- Not enough water for pre-dilution in Kjeldahl determination

BÜCHI Labortechnik AG
Product overview

BUCHI provides Kjeldahl Solutions and support for the entire analytical workflow, from sample preparation to quantification. Depending on sample throughput, varying degrees of instrument integration and automation may be selected.

Sample preparation
Some applications require sample preparation prior to digestion and distillation. With a powerful mixer, non-homogeneous substances can be minced or crushed and homogenized to provide a representative sample.

Digestion
Digestion systems are used to convert nitrogen containing substances (e. g. proteins) in a sample (e. g. milk) into the appropriate form of nitrogen. Beyond classical Kjeldahl digestions, additional applications such as the determination of COD (Chemical Oxygen Demand) can be carried out with the SpeedDigester

Scrubbing
During the digestion process, acid fumes and reaction gases may emerge. A scrubber is connected to the digestion system for neutralizing acid fumes and adsorbing unpleasant odors.

Mixer B-400

SpeedDigester K-425 / K-436

SpeedDigester K-439

KjelDigester K-446

KjelDigester K-449

Scrubber K-415

Distillation

Digested samples can be processed directly in one of BUCHI's high-efficiency distillation units. The range of applications includes classical Kjeldahl tasks such as TKN (Total Kjeldahl Nitrogen) or Devarda as well as the direct distillation of other volatile components such as alcohol, sulfur dioxide, phenol, volatile acids, TVBN (Total Volatile Basic Nitrogen), formaldehyde and cyanide.

Titration

Reflecting different automation requirements, some distillation units actually combine the distillation step with the determination, for example by titration. The product range features an expandable distillation unit with the option of connecting it with a titrator of your choice as well as an automated unit with integrated titration.

Distillation Unit K-350

Distillation Unit K-355

KjelFlex K-360
with external titration

KjelMaster System K-375 / K-376 / K-377
with integrated titration and data management

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With BUCHI affiliates, BUCHI support centers, and several BUCHI distribution partners we cover the globe operated by certified and highly qualified personal, who are regularly trained by BUCHI.

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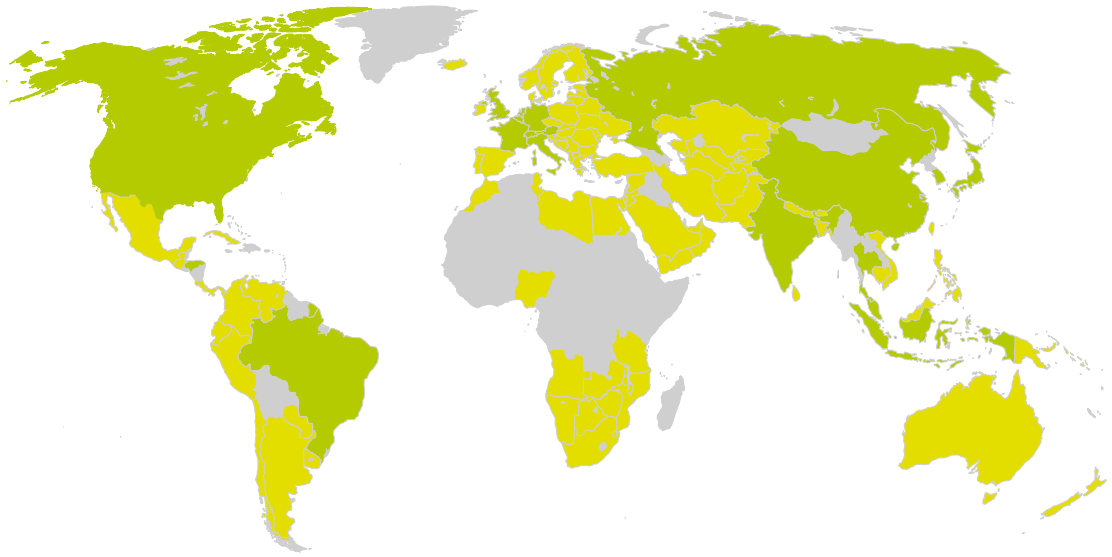

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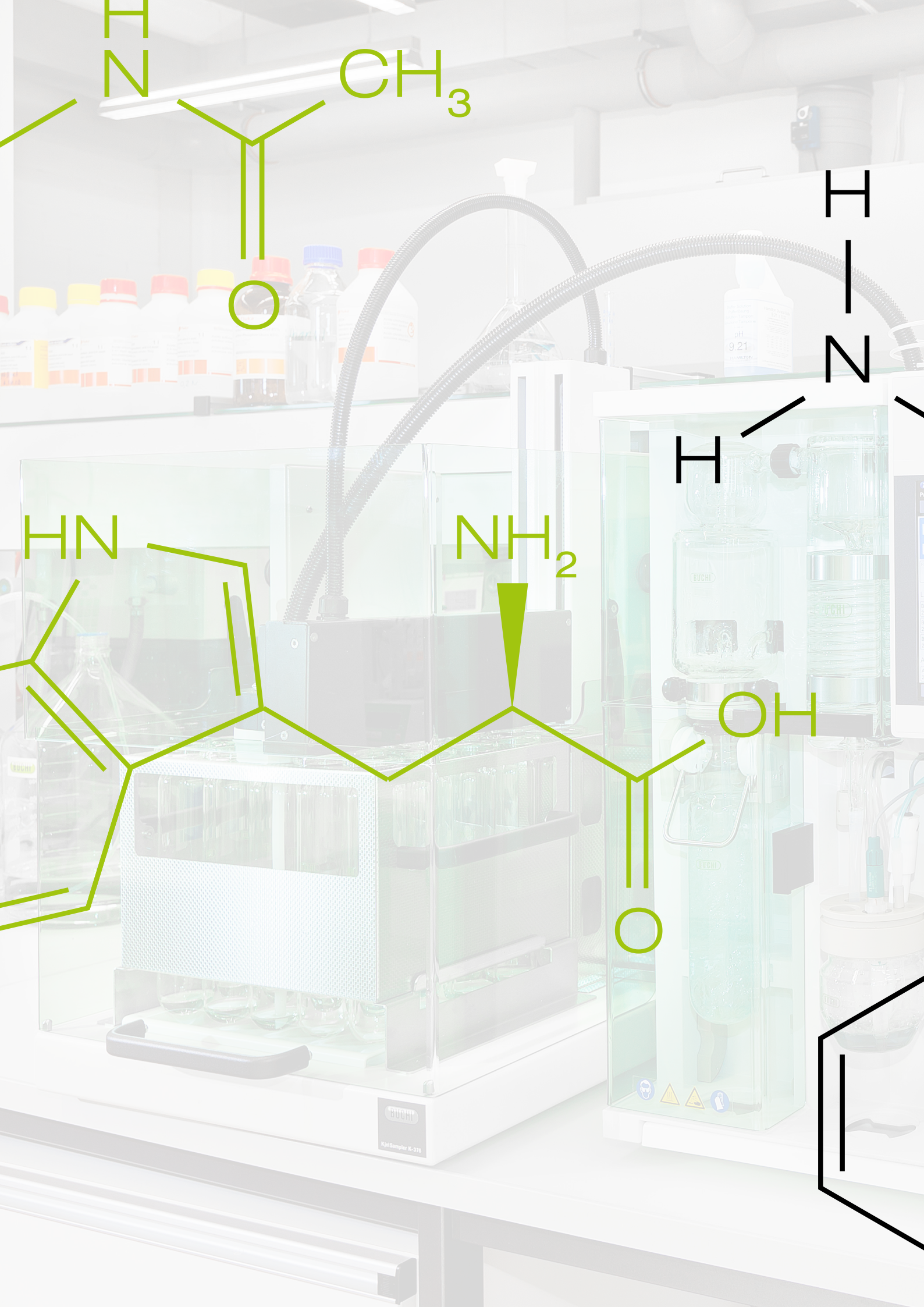
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