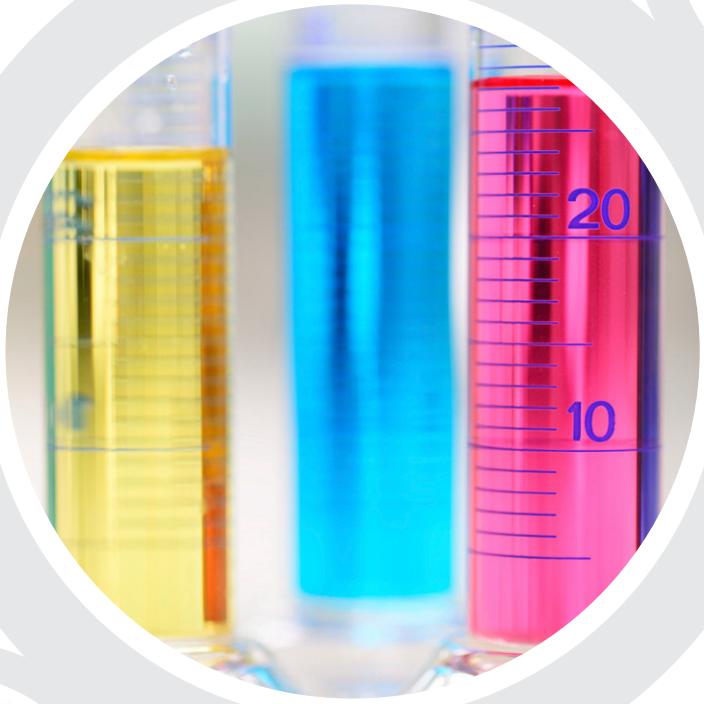
Application Note
No. 252/2016

Selective nitrogen determination methods related to Kjeldahl

KjelMaster System K-375 / K-376, KjelDigester K-449, Scrubber K-415



1. Introduction

85 % of the world's nitrogen demand for fertilizer is primarily derived from ammonia in the form of urea, ammonium nitrate, phosphate and sulfate [1]. Ammoniacal components can also be found in soil, sludge and bio waste. In those sample matrices, a mixture of several nitrogen containing compounds as urea, nitrogen from other organic origin such as amino acids, proteins, nitrate, nitrite and ammonium can be found.

Using the Kjeldahl equipment, selective nitrogen determination of the Total Kjeldahl Nitrogen (TKN and TKN+), the ammonium (ammonium distillation) and the nitrate concentration (Devarda distillation) of a sample is possible. In addition, to these parameters, the organic nitrogen content can be calculated.

Determining the nitrogen sources in mixtures containing urea and / or nitrate is limited. Urea easily reacts with water to form ammonium carbonate. Ammonium carbonate is unstable and decomposes into carbon dioxide and ammonia gas, which is lost to the atmosphere [2 - 4].
In addition, nitrate / nitrite in samples can influence the TKN result. During digestion, nitrate can oxidize a portion of the ammonium released from the digested organic nitrogen, producing volatile N_2O and resulting in a lower recovery rate [5]. For nitrate containing fertilizers the AOAC 955.04 D standard describes a sample digestion method (TKN+) that overcomes the influences of nitrate in samples. For urea and / or nitrate containing samples such as fertilizer, soil, sludge, bio waste and related waste, dedicated nitrogen determination procedures have to be applied.

Here we aim to detail a strategy to selectively determine nitrogenous compounds. An overview of the nitrogen compounds analyzed in this study is shown in Figure 1.

<table>
<tr><td colspan="4">Total Nitrogen (TN)
e.g. amines, amides, amino acids and their derivatives, azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone.</td></tr>
<tr><td colspan="3">Total Kjeldahl Nitrogen+ (TKN+)
e.g. amines, amides, amino acids and their derivatives, nitrate, nitrite.</td><td rowspan="3">Not detectable with Kjeldahl method
Azide, azine, azo, hydrazone, nitrile, nitro, nitroso, oxime, and semi-carbazone.</td></tr>
<tr><td colspan="2">Total Kjeldahl Nitrogen (TKN)
e.g. amines, amides, amino acids and their derivatives.</td><td rowspan="2">Direct distillation
(Distillation without digestion)
Ammonium, nitrate and nitrite can be determined by direct distillation.</td></tr>
<tr><td>Nitrogen from organic origin as; e.g. amines, amides (e.g. urea) and amino acids (proteins).</td><td>Ammonium distillation
e.g. ammonium sulfate, ammonium dihydrogen phosphate, ammonium salts etc.</td><td>Ammonium distillation
e.g. ammonium sulfate, ammonium dihydrogen phosphate, ammonium salts etc.</td><td>Devarda distillation
Nitrate and nitrite.</td></tr>
</table>

Figure 1: Nitrogen compounds which can be determined with methods related to Kjeldahl.

2. Equipment

Equipment for TKN and TKN+ nitrogen determination:

- KjelMaster System K-375 / K-376 with pH electrode
- Sample tubes 300 mL (037377)
- KjelDigester K-449
- Scrubber K-415 TripleScrub^{ECO}
- Analytical balance (accuracy ± 0.1 mg)

Equipment for ammonium determination:

- KjelMaster K-375 with pH electrode
- Sample tubes 500 mL (043982)
- Analytical balance (accuracy ± 0.1 mg)

Equipment for nitrate / nitrite determination (Devarda distillation):

- KjelMaster K-375 with pH electrode
- Devarda splash protector (043335)
- Sample tubes 500 mL (043982)
- Analytical balance (accuracy ± 0.1 mg)

3. Chemicals and Materials

Chemicals for TKN and TKN+ nitrogen determination:

- Sulfuric acid conc. 98 %, Merck (1007482500)
- Kjeldahl Tablet Titanium BUCHI (11057980)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 400 g boric acid, Brenntag (80948-155) diluted to 10 L with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.25 mol/L, Riedel-de Haen (53555)
- Neutralization solution for the Scrubber: 600 g sodium carbonate, calcined, technical, Synopharm (0179420) about 2 mL ethanol and a spatula tip of bromothymol blue, Fluka (18460) diluted to 3 L with distilled water

For TKN+ determination, the following chemicals are used in addition:

- Salicylic acid, Fluka (84215)
- Sodium thiosulfate pentahydrate, SIGMA (31459)

Chemicals for ammonium determination:

- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 400 g boric acid, Brenntag (80948-155) diluted to 10 L with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.25 mol/L, Riedel-de Haen (53555)

Chemicals for nitrate / nitrite determination (Devarda distillation):

- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 400 g boric acid, Brenntag (80948-155) diluted to 10 L with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.25 mol/L, Riedel-de Haen (53555)
- Devarda's alloy (Fluka, 31385)

4. Samples

For TKN, TKN+, ammonium and nitrate determination reference substances were directly analyzed or combined to form model mixtures. All samples were determined in triplicate (n=3).

Sample 1	Glycine (MERCK, 1.04201, ≥ 99.7 %)
Sample 2	Urea (MERCK, 12228387, ≥ 99.5 %)
Sample 3	Ammonium dihydrogen phosphate (Fluka, 09709, ≥ 99.5 %)
Sample 4	Ammonium nitrate (SIGMA, A9642, ≥ 99.0 %)
Sample 5	5 g glycine + 5 g ammonium nitrate
Sample 6	5 g glycine + 5 g sodium nitrate
Sample 7	5 g glycine + 5 g ammonium nitrate + 5 g ammonium dihydrogen phosphate
Sample 8	5 g glycine + 5 g sodium nitrate + 5 g ammonium dihydrogen phosphate

5. Procedure

5.1. Total Kjeldahl Nitrogen (TKN)

The Kjeldahl method depends on the conversion of nitrogen to ammonium by digestion of the organic material with concentrated sulfuric acid, a catalyst and heat. Ammonia is released by distillation and the nitrogen content is determined using boric acid titration.
If the sample contains ammonium e.g. ammonium sulfate, the organic nitrogen and the ammonium nitrogen are determined as a sum parameter. To quantify the organic nitrogen only, the ammonium content must be determined by direct ammonium distillation and then subtracted from the TKN content.

Digestion was performed using the KjelDigester K-449. To predict the optimum digestion parameters, the KjelOptimizer App was used [6]. The determination of TKN in the samples 1 - 8 was performed using the following procedure:

1. Weigh sample into a 300 mL sample tube, prepare additional blanks (without sample).
2. Add two Kjeldahl Tablets Titanium and 15 mL of sulfuric acid (conc. 98 %) to each tube.
3. Start the digestion according to the parameters listed in Table 1. If the liquid inside the sample tube is not clear / blue-green, digest for additional 15 min at 420° C.
4. Let the samples cool down to room temperature.
5. Distill and titrate the samples using the parameters listed in Table 2, starting with the blank samples.

Table 1: Temperature profile for digestion with the K-449.

Step	Temperature [°C]	Time [min]
1	300	0
2	420	90
Cooling	–	35

Table 2: Parameters for TKN distillation and titration with the KjelMaster System K-375 / K-376.

H_2O volume	60 mL	Sensor type	Potentiometric
NaOH (32 %) volume	70 mL	Titration mode	Standard
Reaction time	5 s	Measuring mode	Endpoint pH
Distillation mode	Fixed time	Endpoint pH	4.65
Distillation time	180 s	Stirrer speed titration	7
Stirrer speed distillation	5	Titration start volume	0 mL
Steam output	100 %	Titration algorithm	Optimal
Titration type	Boric acid	Aspiration sample tube	Yes
Receiving solution vol.	50 mL	Aspiration receiving vessel	Yes
Titration solution	H_2SO_4 0.25 mol/L		

5.2. Total Kjeldahl Nitrogen^{+} (TKN+) for nitrate containing samples

The TKN+ method is described in the AOAC 955.04 D standard for nitrate containing fertilizers [7]. When applying the TKN+ method, the nitrate and nitrite in the sample react with sulfuric and salicylic acid in an electrophilic aromatic substitution to form nitrosalicylic acid, this is then reduced with thiosulfate to the amino form (see Figure 2). In the amino form, the nitrogen from nitrate and nitrite is co-determined with the organic nitrogen and the ammonium from inorganic origin.
With this method, the organic nitrogen, nitrate / nitrite and the inorganic ammonium nitrogen are determined in one experiment.

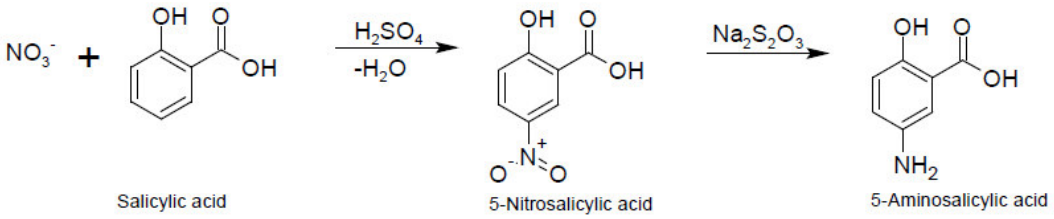


Figure 2: Reaction of nitrate-nitrogen to amino-nitrogen.

To quantify the organic nitrogen only, the ammonium and nitrate content must be determined separately using direct Ammonium distillation and Devarda distillation.
The TKN+ method is not applicable to samples with a high Cl : NO_3^- ratio or to liquid fertilizers because the water content inhibits the reaction from nitrate to nitrosalicylic acid. However, tests showed that the TKN+ method works for sample weights of up to 1 g liquid fertilizers which is sufficient for reproducible results. Higher sample weights lead to lower recoveries of nitrogen from nitrate. For further information, please read the Application Note 029/2010 [8].

Digestion was performed using the KjelDigester K-449. The determination of TKN+ in the reference samples was performed using the following procedure:

1. Weigh sample into a 300 mL sample tube, prepare additional blanks (without sample).
2. Add one Kjeldahl Tablet Titanium and 20 mL of sulfuric acid (conc. 98 %) to each tube.
3. Add approximately 1 g of salicylic acid to each sample tube.
4. Swirl until thoroughly mixed and let stand >30 min at room temperature.
5. Add approximately 2.5 g sodium thiosulfate pentahydrate, swirl and let stand for 5 min. at room temperature.
6. Start the digestion using the parameters listed in Table 1. If the liquid inside the sample tubes is not clear / blue-green, digest for additional 15 min at 420° C.
7. Let the samples cool down to room temperature.
8. Distill and titrate the samples using the parameters listed in Table 2, starting with the blank samples.

5.3. Ammonium distillation

Ammoniacal nitrogen from inorganic sources such as ammonium sulfate or ammonium dihydrogen phosphate, is quantified by direct ammonium distillation, Alkalization of the sample followed by direct steam distillation and boric acid titration without prior digestion is necessary. Alkalization with sodium hydroxide converts the ammonium (NH_4^+) to volatile ammonia (NH_3).

For samples that contain both, ammonium and nitrate (NO_3^-), first ammoniacal nitrogen is determined by direct ammonium distillation, followed by the Devarda distillation. To predict the optimum sample weights, the KjelOptimizer App was used [6].

The ammonium distillation in reference samples was determined as follows:

1. Weight each sample into a 500 mL sample tube.
2. Connect the sample tube to the K-375 distillation unit.
3. Distill and titrate the sample using the parameters listed in Table 3. Start with the blank samples.

Table 3: Parameters for ammonium distillation and titration using the KjelMaster K-375.

H_2O volume	10 mL	Sensor type	Potentiometric
NaOH (32%) volume	30 mL	Titration mode	Standard
Reaction time	5 s	Measuring mode	Endpoint pH
Distillation mode	Fixed time	Endpoint pH	4.65
Distillation time	200 s	Stirrer speed titration	7
Stirrer speed distillation	5	Titration start volume	0 mL
Steam output	100 %	Titration algorithm	Optimal
Titration type	Boric acid	Aspiration sample tube	**No***
Receiving solution vol.	50 mL	Aspiration receiving vessel	Yes
Titration solution	H_2SO_4 0.25 mol/L		

***Important: After distillation, place the distilled sample back into a sample rack and let it cool down to ambient temperature. After cooling down, the nitrate determination using Devarda distillation (described below) is performed using the same sample.

5.4. Nitrate / Nitrite determination (Devarda distillation)

Devarda's alloy (50 % Cu, 45 % Al, and 5 % Zn) reduces nitrate and nitrite to ammonium (NH_4^+) in alkaline conditions. Hence, the nitrogen from nitrate / nitrite is steam distillable and can be determined by boric acid titration as in the Kjeldahl method. To save time and sample, the already distilled sample from the ammonium distillation (see section 5.3.) can be reused for nitrate determination.
If samples (without prior ammonium distillation) are treated with Devarda`s alloy, the nitrogen from nitrate and ammonium from the sample will be determined. To quantify the nitrate / nitrite nitrogen amount only, the ammonium content must be measured in addition by direct distillation without Devarda's alloy and subtracted from the result.

The procedure for nitrate determination was:

1. Add ± 2.00 g Devarda's alloy to the cooled sample tube from the ammonium determination. Devarda's alloy is added immediately before distillation. Do not add the Devarda's alloy in advance to the sample tube as the reaction starts immediately after addition.
2. Connect the sample tube to the KjelMaster K-375.
3. Distill and titrate the samples using the parameters listed in Table 4. Start with the blank samples.

Table 4: Parameters for nitrate / nitrite distillation and titration with the KjelMaster K-375.

H_2O volume	0 mL	Sensor type	Potentiometric
NaOH (32%) volume	20 mL	Titration mode	Standard
Reaction time	300 s	Measuring mode	Endpoint pH
Distillation mode	Fixed time	Endpoint pH	4.65
Distillation time	300 s	Stirrer speed titration	7
Stirrer speed distillation	5	Titration start volume	0 mL
Steam output	100 %	Titration algorithm	Optimal
Titration type	Boric acid	Aspiration sample tube	No
Receiving solution vol.	50 mL	Aspiration receiving vessel	Yes
Titration solution	H_2SO_4 0.25 mol/L		

6. Results and Discussion

6.1. Total Kjeldahl Nitrogen (TKN)

Table 5 shows the theoretical total nitrogen of the sample [%], the expected Total Kjeldahl Nitrogen content (organic nitrogen and inorganic sourced ammonium) [%], the measured N content [%] with standard deviation, the relative standard deviation (RSD) and the recovery rate (RR) on TKN [%]. The mean blank volume (V_{Blank}) was 0.101 mL (n=3).

Table 5: Results of the Total Kjeldahl Nitrogen (TKN) determination.

Chemical constitution	Total Nitrogen w/w [%]	Total Kjeldahl Nitrogen w/w [%]	Measured N [%]	RSD [%]	RR on TKN [%]
Glycine	18.66	18.66	18.56 ± 0.01	0.06	**99.45**
Urea	46.65	46.65	46.44 ± 0.01	0.02	**99.53**
Ammonium dihydrogen phosphate	12.18	12.18	12.09 ± 0.01	0.08	**99.26**
Ammonium nitrate	35.01	17.50	9.19 ± 0.14	1.54	**52.54**
1/2 glycine + 1/2 ammonium nitrate	26.83	18.08	12.06 ± 0.10	0.81	**66.69**
1/2 glycine + 1/2 sodium nitrate	17.57	9.33	4.00 ± 0.18	4.47	**42.92**
1/3 glycine + 1/3 ammonium nitrate + 1/3 ammonium dihydrogen phosphate	21.95	16.12	12.23 ± 0.13	1.03	**75.84**
1/3 glycine + 1/3 sodium nitrate + 1/3 ammonium dihydrogen phosphate	15.78	10.28	6.31 ± 0.25	4.01	**61.40**

With the TKN method, nitrogen from amines, amides, amino acids and their derivatives are determined. Nitrogen sources such as nitrate are not measured with TKN method (see Figure 1). Due to the interfering properties of nitrate with ammonium, all nitrate containing samples showed low recovery rates on the Total Kjeldahl Nitrogen content. Nitrogen from organic / inorganic ammonium origin was lost during digestion when nitrate was present. Thus, observed recovery rates for nitrate containing samples are far below the calculated recovery rates based on TKN. Therefore, the method TKN+ must be applied for nitrate containing samples (see section 6.2.).

In samples that do not contain nitrate (glycine, urea and ammonium dihydrogen phosphate) very good results with recoveries >99 % were achieved.

The digestion step before distillation, converts nitrogen from organic origin to ammonium. When a sample also contains ammonium of inorganic origin, e.g. ammonium dihydrogen phosphate, both the ammonium from organic and inorganic origin are co-distillated using the TKN method.

To determine the inorganic ammonium (ammonium dihydrogen phosphate) only, a further ammonium determination without digestion is necessary (ammonium distillation). The difference between TKN and ammonium distillation will reveal the organic nitrogen content of the sample (e.g. glycine).

6.2. Total Kjeldahl Nitrogen for nitrate containing samples (TKN+)

Table 6 shows the expected total nitrogen, the measured N, the RSD and the RR. The mean blank volume (V_{Blank}) was 0.119 mL (n=3).

Table 6: Results of the Total Kjeldahl Nitrogen (TKN+) determination.

Chemical constitution	Total Nitrogen w/w [%]	Measured N [%]	RSD [%]	RR on TN [%]
Glycine	18.66	18.18 ± 0.02	0.14	**99.01**
Urea	46.65	46.48 ± 0.02	0.04	**99.62**
Ammonium dihydrogen phosphate	12.18	12.07 ± 0.03	0.23	**99.09**
Ammonium nitrate	35.01	34.74 ± 0.12	0.35	**99.24**
1/2 glycine + 1/2 ammonium nitrate	26.83	26.59 ± 0.09	0.34	**99.09**
1/2 glycine + 1/2 sodium nitrate	17.57	17.23 ± 0.05	0.30	**98.07**
1/3 glycine + 1/3 ammonium nitrate + 1/3 ammonium dihydrogen phosphate	21.95	21.67 ± 0.11	0.49	**98.70**
1/3 glycine + 1/3 sodium nitrate + 1/3 ammonium dihydrogen phosphate	15.78	15.47 ± 0.03	0.17	**98.03**

Applying the TKN+ method, the nitrogen bound in nitrate is co-determined. With the TKN+ digestion, all samples fulfilll the recovery requirements of the Total Nitrogen (98 % - 102 %) [9]. Therefore, for nitrate containing samples, TKN+ determination is required. The TKN+ digestion method showed no influence on recovery rates of organic samples (glycine and urea) or ammonium only (ammonium dihydrogen phosphate) samples.

6.3. Ammonium distillation

In Table 7 the results of the direct ammonium distillation are listed. The expected nitrogen content is calculated only from the ammonium content in the sample. V_{Blank} = 0.101 mL (n=3).

Table 7: Results of the ammonium nitrogen determination.

Chemical constitution	N from ammonium [%]	Measured N [%]	RSD [%]	RR [%]
Glycine	0.00	0.00 ± 0.0	-	**-**
Urea	0.00	2.10 ± 0.05	2.36	**Too high***
Ammonium dihydrogen phosphate	12.18	12.12 ± 0.01	0.08	**99.49**
Ammonium nitrate	17.50	17.43 ± 0.05	0.26	**99.62**
1/2 glycine + 1/2 ammonium nitrate	8.75	8.73 ± 0.04	0.47	**99.72**
1/2 glycine + 1/2 sodium nitrate	0.00	0.00 ± 0.00	-	**-**
1/3 glycine + 1/3 ammonium nitrate + 1/3 ammonium dihydrogen phosphate	9.89	9.84 ± 0.04	0.39	**99.50**
1/3 glycine + 1/3 sodium nitrate + 1/3 ammonium dihydrogen phosphate	4.06	4.08 ± 0.04	0.88	**100.41**

* Urea is unstable and easily breaks down into carbon dioxide and ammonia gas, which is distilled and detected by boric acid titration even without a prior digestion step.

NOTE: In a sample matrix containing urea, direct distillation methods such as ammonium distillation, lead to false positive ammonium results due to the breakdown of the urea by steam distillation. Thus, the inorganic ammonium can't be determined in urea containing sample matrixes.
Other organic components such as amino acids / proteins won't be affected by the distillation process. No nitrogen was detected when the amino acid glycine was distilled directly. However, all samples (except urea) fulfill the recovery requirements of 100 ±2 % [9].

6.4. Nitrate / Nitrite determination (Devarda distillation)

Table 8 shows the results of the expected nitrogen content from nitrate / nitrite origin, the measured N content, the RSD and the RR of the nitrate nitrogen determination (Devarda distillation). The mean blank volume (V_{Blank}) was 0.156 mL (n=3).

Table 8: Results of the nitrate nitrogen determination (Devarda distillation).

Chemical constitution	N from nitrate / nitrite origin [%]	Measured N [%]	RSD [%]	RR [%]
Glycine	0.00	0.00 ± 0.0	-	-
Urea	0.00	7.68 ± 1.12	14.59	**Too high***
Ammonium dihydrogen phosphate	0.00	0.00 ± 0.00	0.00	-
Ammonium nitrate	17.50	17.34 ± 0.15	0.87	**99.10**
1/2 glycine + 1/2 ammonium nitrate	8.75	8.71 ± 0.06	0.65	**99.53**
1/2 glycine + 1/2 sodium nitrate	8.24	8.17 ± 0.06	0.71	**99.11**
1/3 glycine + 1/3 ammonium nitrate + 1/3 ammonium dihydrogen phosphate	5.83	5.93 ± 0.05	0.78	**101.74**
1/3 glycine + 1/3 sodium nitrate + 1/3 ammonium dihydrogen phosphate	5.49	5.42 ± 0.05	1.00	**98.71**

* Urea is unstable and easily breaks down into carbon dioxide and ammonia gas, which is distilled and detected by boric acid titration even without a prior digestion step. Thus, the nitrate / nitrite content can't be determined in urea containing sample matrixes.

Organic components such as amino acids / proteins won't be affected by the Devarda distillation method. The ammonium dihydrogen phosphate sample was directly distilled using the ammonium distillation method first. Hence, no ammonium could be determined with Devarda distillation.
The analysed samples (except urea sample) fulfill the recovery requirements of 100 ±2 % [9].

7. Conclusion

The Kjeldahl method is applicable to amines, amides, amino acids and their derivatives but generally fails to give quantitative results when nitrogen is in N=N and N–O linkages [10]. Nevertheless, the nitrate (NO_3^-) and nitrite (NO_2^-) content is included in the result, when the TKN+ method according to AOAC 955.04D is applied [7, 8, 11]. With the Devarda distillation, NO_3^- and NO_2^- are selectively determined [8, 10].

To determine nitrogen in nitrate and/or urea containing samples using Kjeldahl requires a special determination procedure due to interferences with the sample matrix. During Kjeldahl digestion, nitrate can oxidize a portion of the ammonium released from the digested organic nitrogen, producing N_2O and resulting in negative interference [5]. When applying the TKN+ digestion according to AOAC 955.04D, the interferences from the nitrate can be eliminated.

In urea containing samples, direct distillation methods such as ammonium distillation or Devarda distillation, lead to false positive ammonium and nitrate results due to the breakdown of the urea by steam heat. Urea containing samples can be analyzed by TKN or TKN+ determination only.
Depending on the sample matrix, different determination methods are applicable. A decision tree was created, which shows the determination methods applicable for different sample matrixes (Page 11).

8. References

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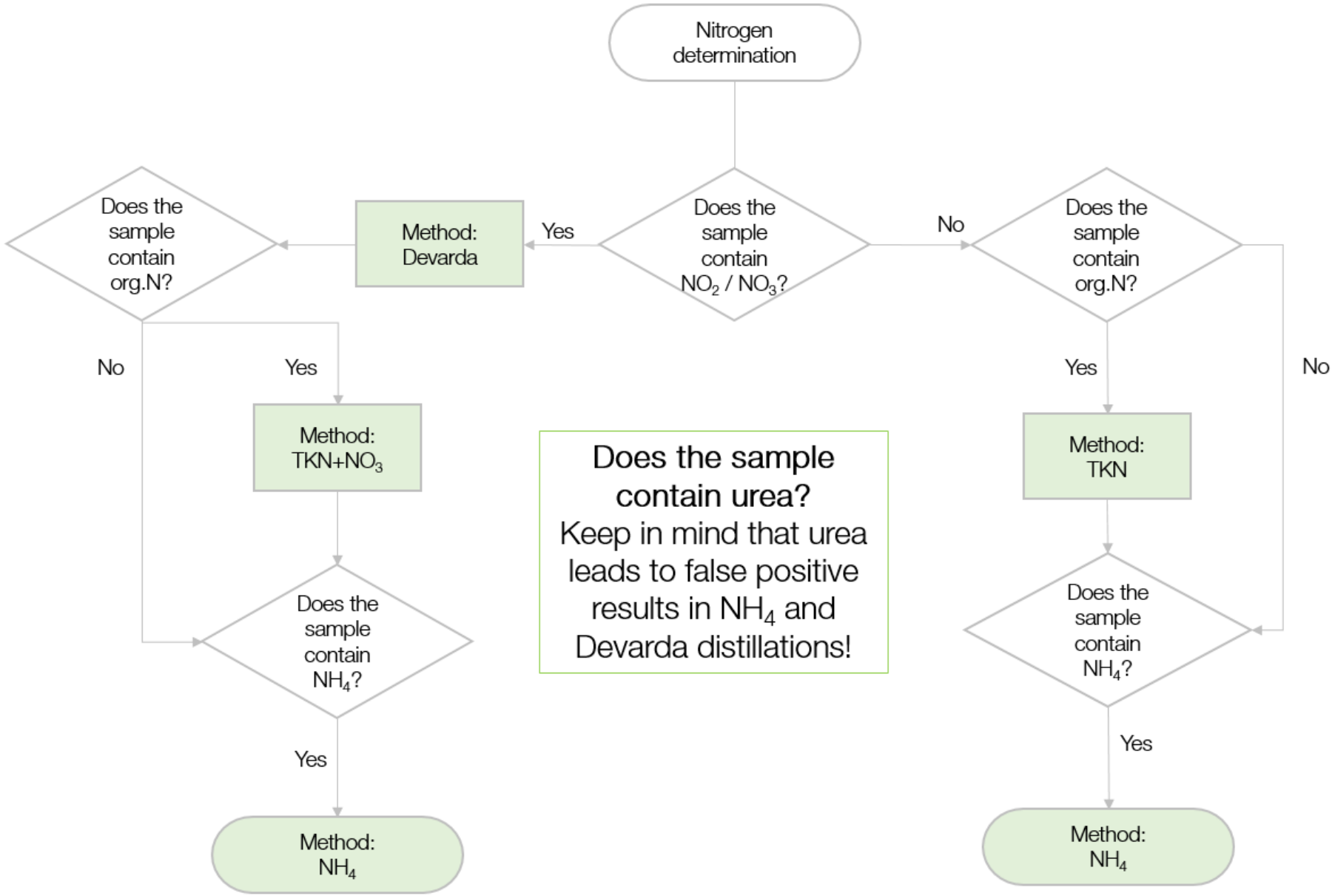

Decision tree, showing the determination methods applicable for different sample matrixes.