Application Note

100/2013

Digest Automat K-438

KjelMaster K-375 with KjelSampler K-376

Nitrogen Determination in Reference Substances – Operational Quality Check

Nitrogen Determination in Reference Substances – Operational Quality Check Procedure

A simple method to test the performance of the Kjeldahl equipment according to the EN ISO 8968-2:2001, AOAC 991.20 or AOAC 2001.11 regulations is introduced below. The reference substances, with specific nitrogen content are digested with sulfuric acid and Kjeldahl Tablets Titanium using the Digest Automat K-438, followed by distillation and titration using the KjelMaster K-375 with KjelSampler K-376.

Introduction

In order to verify the BUCHI Digest System K-437 or the Digest Automat K-438 in combination with the KjelMaster K-375 with KjelSampler K-376, the nitrogen content of different reference substances is determined according to the classical Kjeldahl method. All recoveries of the samples should be within the specification of 98-102%.

Experimental

Instrumentation:

Digest Automat K-438
KjelMaster K-375 with KjelSampler K-376

Reference substances:

Glycine, DL-tryptophan and Acetanilide

Determination:

The substances were added directly into a sample tube as described in Table 1. A portion of 15 ml sulfuric acid and two Titanium tablets were added, and the digestion was performed using the parameters specified in Table 2. After digestion the ammonia of the sample was distilled into a boric acid solution by steam distillation and titrated with sulfuric acid (Table 3).

Table 1: Weight for each reference substance

Reference substance	Weight [g]
Glycine	0.11
Tryptophan	0.14
Acetanilide	0.17

The samples are digested according to the parameters listed in Table 2.

Table 2: Temperature profile for digestion with the K-438

Step	Temp. [°C]	Time [min]
Preheat	320	-
1	420	120
Cooling	-	30

After cooling down the samples to ambient temperature, the samples are distilled and titrated with the KjelMaster K-375 and KjelSampler K-376 (Table 3).

Table 3: Parameters for distillation and titration with the KjelMaster K-375 and KjelSampler K-376

Method Parameters			
H_2O volume	50 ml	Steam output	100 %
NaOH volume	60 ml	Receiving solution	50 ml H_3BO_3 4%
Reaction time	5 s	Titration solution	H_2SO_4 0.1 mol/l
Dist. mode	Fixed time	Endpoint pH	4.65
Dist. time	180 s	Stirrer sp. titr.	7
Stirrer sp. dist.	5	Titr. algorithm	Optimal

Results

The determined recoveries of the reference substances are presented in Table 4.

Table 4: Determined recoveries of the reference substances

Reference substance	Recovery [%]	RSD [%] (n=4)
Glycine	99.6	0.1
Tryptophan	99.0	0.5
Acetanilide	99.5	0.1

Acetanilide and tryptophan are mentioned in different official methods like AOAC 920.11 or AOAC 2001.11. In these regulations a recovery of 98-102% is defined to evaluate the success of the digestion. Taking a look at the results presented in Table 4 shows that the recoveries are within the requested specification.

Conclusion

The determination of nitrogen recovery in the reference substances tryptophan, acetanilide and glycine using the K-438 and KjelMaster K-375 with KjelSampler K-376, provides a reliable check procedure to test the performance of the whole Kjeldahl process.

References

EN ISO 8968-2:2001
AOAC 991.20
AOAC 2001.11

Operation manual DigestAutomat K-432/ K-438
Operation manual KjelMaster K-375 with KjelSampler K-376

For more detailed information and safety considerations please refer to Application Note 100/2013.

1 Introduction

In order to verify the Digest System K-437 or the Digest Automat K-438, respectively, in combination with the KjelMaster K-375 with KjelSampler K-376 the nitrogen content of different reference substances are determined by the Kjeldahl method. To obtain significant results, at least 3 blanks and 3 reference samples must be analysed. The samples are digested using the Digest Automat K-438. The distillation and boric acid titration are performed using the KjelMaster K-375 with KjelSampler K-376.

2 Equipment

- Digest Automat K-438 (the parameters used are also valid for K-437)
- Scrubber K-415 TripleScrub ^{ECO}
- KjelMaster K-375 with KjelSampler K-376 (KjelSampler K-377 is also possible)
- Analytical balance (accuracy ± 0.1 mg)

3 Chemicals and Materials

- Sulfuric acid, conc. 98 %, Fluka (84727)
- Titanium, BUCHI Kjeldahl Tablet (11057980)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 200 g of boric acid, Brenntag (80948-155) diluted to 5 l with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.1 mol/l, Fluka (35357) standard solution
- Neutralization solution for the Scrubber: 600 g of sodium carbonate, calcined, technical, Synopharm (0179420) about 2 ml of ethanol and a spatula tip of bromthymol blue, Fluka (18460) diluted to 3 l with distilled water

For safe handling please pay attention to all corresponding MSDS data sheets!

Reference substances

- Glycine, Merck (1.04201; assay: >99.7 %))
- DL-tryptophan, Alfa Aesar (L05936; assay: > 99 %)
- Acetanilide, Aldrich (112933; assay: >99 %))

4 Procedure

4.1 Digestion method

- Preheat the Digest Automat K-438 according to the parameters listed in Table 1

Table 1: Temperature profile for digestion with the K-438

Step	Temperature [°C]	Time [min]
Preheating	320	-
1	420	120
Cooling	-	30

- Place each sample into a 300 ml sample tube, according to Table 2
- Add two Titanium Tablets and 15 ml sulfuric acid (98 %)
- Prepare additional blanks
- Carefully suspend the sample by gently swirling the tube
- Connect the Scrubber K-415 to the Digest Automat K-438 for absorbing the acid fumes created during the digestion
- Insert the rack containing the samples into the lift and immediately start the digestion according to the parameters listed in Table 1

Table 2: Weight for each reference substance

Reference substance	Weight [g]
Glycine	0.11
Tryptophan	0.14
Acetanilide	0.17

- If the liquid inside the sample tube is not clear and blue-green, digest for an additional 30 min at 420 °C.

- Let the samples cool down

NOTE: The samples should be clear-green immediately after the digestion.

4.2 Distillation and titration

Distill the samples according to the parameters listed in Table 3

Table 3: Distillation and titration using the KjelMaster K-375 with KjelSampler K-376

Method parameters KjelMaster K-375			
H_2O volume	50 ml	Titration solution	H_2SO_4 0.1 mol/l
NaOH volume	60 ml	Sensor type	Potentiometric
Reaction time	5 s	Titration mode	Standard
Distillation mode	Fixed time	Measuring mode	Endpoint pH
Distillation time	180 s	Endpoint pH	4.65
Stirrer speed distillation	5	Stirrer speed titration	7
Steam output	100 %	Titration start volume	0 ml
Titration type	Boric acid	Titration algorithm	Optimal
Receiving solution vol.	50 ml		

4.3 Calculation

The results are calculated as percentage of nitrogen. The following equations (1), and (2) are used to calculate the results.

$$w_N = \frac{(V_{Sample} - V_{Blank}) \cdot z \cdot c \cdot f \cdot M_N}{m_{Sample} \cdot 1000} \qquad (1)$$

$$\%N = w_N \cdot 100\ \% \qquad (2)$$

w_N	: weight fraction of nitrogen
V_{Sample}	: amount of titrant for the sample [ml]
V_{Blank}	: mean amount of titrant for the blank [ml]
z	: molar valence factor (1 for HCl, 2 for H_2SO_4)
c	: titrant concentration [mol/l]
f	: titrant factor (for commercial solutions normally 1.000)
M_N	: molecular weight of nitrogen (14.007 g/mol)
m_{Sample}	: sample weight [g] (consider the assay of the reference substance)
1000	: conversion factor [ml/l]
%N	: percentage of weight of nitrogen

5 Results

5.1 Recovery of glycine

The results of the nitrogen determination and recovery in glycine are presented in Table 4. The nominal value of glycine (assay: ≥ 99.7 %) is 18.66 % nitrogen (the value corrected with the assay is 18.60 % nitrogen). The recoveries are above the specification of ≥ 98 % [1], [2], [3], [4].

Table 4: Results for the recovery of nitrogen in glycine

Glycine	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.1075	7.297	18.53	99.6
Sample 2	0.1039	7.071	18.56	99.8
Sample 3	0.1082	7.339	18.51	99.5
Sample 4	0.1089	7.391	18.53	99.6
Average	**-**	**-**	**18.53**	**99.6**
Rsd [%]	**-**	**-**	**0.1**	**0.1**

The mean blank volume for this sample was 0.188 ml (n = 4; rsd 0.9%).

5.2 Recovery of tryptophan

The results of the nitrogen determination and recovery in tryptophan are presented in Table 5. The nominal value of tryptophan (assay: ≥ 99.0 %) is 13.72 % nitrogen (the value corrected with the assay is 13.58 % nitrogen). The recoveries are above the specification of ≥ 98 % [1], [2], [3], [4].

Table 5: Results for the recovery of nitrogen in tryptophan

Tryptophan	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.1483	7.339	13.51	99.5
Sample 2	0.1417	6.952	13.37	98.5
Sample 3	0.1498	7.396	13.48	99.2
Sample 4	0.1422	7.000	13.42	98.8
Average	**-**	**-**	**13.45**	**99.0**
Rsd [%]	**-**	**-**	**0.5**	**0.5**

The mean blank volume for this sample was 0.188 ml (n = 4; rsd 0.9%).

5.3 Recovery of acetanilide

The results of the nitrogen determination and recovery in acetanilide are presented in Table 5. The nominal value of acetanilide (assay: 99 %) is 10.36 % nitrogen (the value corrected with the assay is 10.26 % nitrogen). The recoveries are above the specification of ≥ 98 % [1], [2], [3], [4].

Table 5: Results for the recovery of nitrogen in acetanilide

Acetanilide	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.1746	6.554	10.21	99.6
Sample 2	0.1733	6.504	10.21	99.5
Sample 3	0.1752	6.579	10.22	99.6
Sample 4	0.1773	6.638	10.19	99.4
Average	**-**	**-**	**10.21**	**99.5**
Rsd [%]	**-**	**-**	**0.1**	**0.1**

The mean blank volume for this sample was 0.188 ml (n = 4; rsd 0.9%).

6 Conclusion

The presented method shows an easy and effective way to check the complete nitrogen determination of the digestion process with Digest Automat K-438 and the distillation and titration process with KjelMaster K-375 in combination with KjelSampler K-376. The reference substances glycine, tryptophan and acetanilide showed good recovery rates within the specification of ≥ 98% [1; 2; 3] with low relative standard deviation.

The most important characteristics of the latest version of the fully-automatic KjelMaster System K-375/K-376, are the convenient and intuitive operation, short process time as well as sophisticated data management. In combination with the Digest Automat K-438, personnel attendance during sample analysis is significantly reduced.

7 References

[1] DIN EN ISO 8968-2:2001

[2] LFGB §64 L01.00-10/2

[3] AOAC 991.20 Part “Block Digestor/Steam Distillation Method”

[4] AOAC 2001.11 Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain and Oilseed

Operation manual DigestAutomat K-432/ K-438

Operation manual Scrubber K-415

Operation manual KjelMaster K-375 with KjelSampler K-376/K-377

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