



### Challenge

Standard-compliant  $TN_b$  determination in water samples and meeting the required recovery rates for the system test with nicotinic acid

### Solution

Standard compliant  $TN_b$  routine analysis in any environmental water matrix by high temperature multi N/C x300 analyzers

### Intended audience

Industrial water and wastewater laboratories, municipal and industrial wastewater treatment plants, water utilities, environmental authorities, and contract labs for environmental testing

## $TN_b$ Determination in Water Samples for Environmental Monitoring in Accordance with DIN EN ISO 20236

### Introduction

The parameter total bound nitrogen ( $TN_b$ ) is an established parameter in water analysis. It is used to assess and monitor the quality of a wide variety of waters, with wastewater and surface water analysis being the main areas of application.

The international standard DIN EN ISO 20236<sup>1)</sup> describes a catalytic high temperature method for simultaneous determination of TOC (total organic carbon) and  $TN_b$  as well as DOC (dissolved organic carbon) and  $DN_b$  (dissolved bound nitrogen). This new standard replaces the previous DIN EN 12260<sup>2)</sup> for  $TN_b$  determination in Europe.

The method for determining  $TN_b$  in accordance with DIN EN ISO 20236 is based on catalytically assisted high-temperature oxidation of the water sample at temperatures  $\geq 720$  °C in an oxygen-rich atmosphere. The subsequent detection of the NO formed in this process is carried out using a chemiluminescence detector (CLD) or an alternative detector, such as the electrochemical detector described in annex C of the standard, short form: ChD (chemodetector).

Before determining the  $TN_b$  in an unknown water sample, the analyzer used must be calibrated with nitrogen standards of different concentrations. For this purpose, according to the standard, nitrogen mixed standard solutions of potassium nitrate and ammonium sulphate are used. Two stock solutions of  $KNO_3$  and  $(NH_4)_2SO_4$ , each containing 1000 mg/L N, are first prepared. From this, a mixed standard stock solution is prepared by mixing equal volumes (1:1) of the two individual N stock solutions. To prepare the calibration solutions, this mixed standard stock solution is diluted accordingly with ultrapure water. Usually, the  $TN_b$  analyzers are calibrated in a range between 0 and 20 mg/L N, sometimes the calibration range is extended to 50 mg/L. Furthermore, DIN EN ISO 20236 prescribes a daily system test. This is carried out using at least two nicotinic acid standards, whereby the N concentrations should cover the working range. The nicotinic acid standards

for the system test are also prepared from a stock solution containing 1,000 mg/L N by dilution with ultrapure water. The system test is considered passed under the following conditions:

- a) If the measured value does not deviate from the theoretical value by more than  $\pm 5\%$  and/or  $\pm 1$  mg/L (whichever is higher)
  - b) If the repeatability coefficient of variation from at least 2 injections of the nicotinic acid standards does not exceed 5% or  $\pm 1$  mg/L (whichever is higher)
  - c) The individual values must not deviate from each other by more than 1 mg/L for concentrations  $< 10$  mg/L
- DIN EN ISO 20236 also describes interferences that can have a negative effect on the determination of TN<sub>b</sub>.

## Materials and Methods

The TN<sub>b</sub> determinations were carried out on the multi N/C 3300 TOC/TN<sub>b</sub> analyzer. Even though the focus was on the determination of TN<sub>b</sub>, a combined NPOC/TN<sub>b</sub> method was chosen, as this method for the simultaneous determination of organic carbon and total bound nitrogen is used for the majority of samples to be measured in routine laboratories. The acidification required for this type of TOC/TN<sub>b</sub> determination can either be carried out manually in advance (often during sampling) or automatically using an autosampler directly before the actual measurement. The acidified samples are then purged automatically with a partial flow of the carrier gas used. This process removes the TIC (total inorganic carbon in the form of carbonates/hydrogen carbonates) from the samples. The completeness of the TIC removal can be checked automatically by activating the TIC control measurement in an NPOC method. After TIC removal, the sample is injected directly into the combustion tube of the analyzer, which is filled with catalyst. The nitrogen compounds and organic carbon compounds contained in the sample are completely oxidized at high temperatures. The NO formed in this process is fed into a chemiluminescence detector or a chemodetector, while the carbon dioxide formed is fed into the FR-NDIR detector (Focus Radiation Non-Dispersive Infrared Detector). For the automated TN<sub>b</sub>/NPOC determination, the AS vario autosampler was used in combination with a tray of 72 positions for 40 mL vials.

The following influences are named:

1. High levels of TOC/DOC in the sample can lead to reduced recovery of nitrogen. This can be counteracted by measuring TN<sub>b</sub> in different sample dilutions or using the standard addition method.
2. Nitrogen compounds with double or triple bonds are not always completely oxidized to NO.
3. The described calibration with the mixed standard solution of equal parts of potassium nitrate and ammonium sulphate can lead to a positive bias for nitrate-N determinations (e.g., in a KNO<sub>3</sub> solution) and a negative bias for ammonium-N determinations (e.g., in a (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution).

The aim of this application note is to show how the multi N/C 3300 TOC/TN<sub>b</sub> analyzer not only meets the requirements of DIN EN ISO 20236 with regard to nitrogen determination, but also convinces with very good performance characteristics. For this purpose, both standards and samples with different compositions were analyzed.

### Samples and reagents

- Nitrogen mixed standard calibration solutions in the concentration range 1 mg/L to 50 mg/L, produced from KNO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>
- Nicotinic acid solutions for the system test, concentrations 5 mg/L, 10 mg/L, 20 mg/L, and 50 mg/L N
- Control standard solutions of potassium nitrate and ammonium sulphate, ratio NO<sub>3</sub>-N to NH<sub>4</sub>-N = 1:1, concentrations 5 mg/L, 10 mg/L, 20 mg/L, and 50 mg/L N
- Control standard solutions of potassium nitrate, 10 mg/L and 50 mg/L N
- Control standard solutions of ammonium sulphate, 10 mg/L and 50 mg/L N
- 2 mol/L HCl to acidify the samples and standard solutions
- 5 water samples (1 surface water, 2 process water, 2 municipal wastewater): Samples A to E

### Sample preparation

All samples were acidified with 2 mol/L HCl (0.5 mL per 100 mL sample) and stored in the refrigerator at approx. 4 °C until measurement. After appropriate warming to room temperature, the samples were filled into 40 mL sample vials and placed on the tray of the autosampler.

All calibration and control standard solutions as well as the nicotinic acid solutions for the system test were freshly prepared from their corresponding stock solutions on the day of measurement.

## Calibration

The calibration was performed with two different device configurations:

- multi N/C 3300 with chemiluminescence detector (CLD)
- multi N/C 3300 with chemodetector (ChD)

For both configurations, a multi-point calibration was carried out in the concentration range from 1 to 50 mg/L N in each case. Mixed standard calibration solutions (potassium nitrate and ammonium sulphate in ultrapure water, equal proportions of nitrate-N and ammonium-N) were used for this purpose.

The calibration curves were evaluated using linear regression.

Both calibration curves (for CLD and ChD) and the correlation coefficients obtained are shown in Figures 1 and 2.

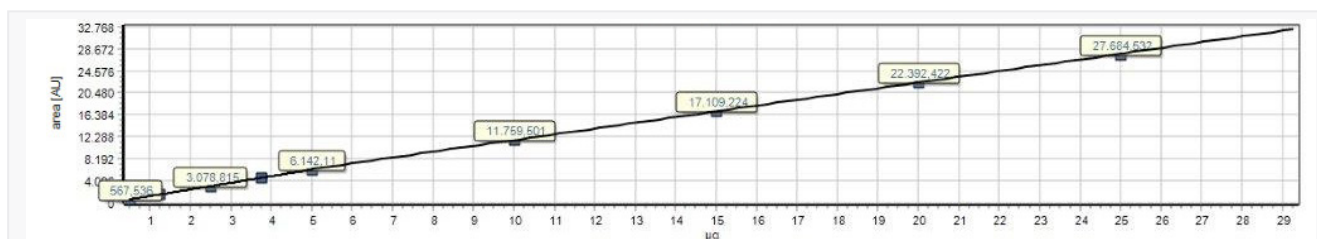


Figure 1: Calibration curve 1–50 mg/L TN<sub>b</sub>,  $R_2 = 0.99981$ , determined with chemiluminescence detector (CLD)

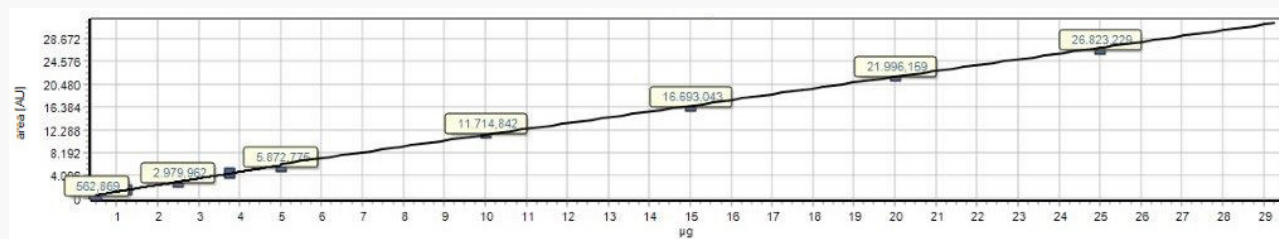


Figure 2: Calibration curve 1–50 mg/L TN<sub>b</sub>,  $R_2 = 0.99996$ , determined with chemodetector (ChD)

Table 1: Device and method settings for the standard and sample measurements

Parameter	Settings for multi N/C 3300
Method	TN <sub>b</sub> /NPOC with TIC control
Digestion method	High-temperature combustion with platinum catalyst
Digestion temperature	800 °C
Carrier gas	Synthetic air (free of CO <sub>2</sub> and hydrocarbons)
Number of repeat measurements per vessel	min. 3, max. 4
Autosampler, tray and vessel sizes	AS vario, 72 pos. tray, 40 mL vials
Number of rinsing cycles with sample before the 1st injection	3
Number of rinsing cycles with ultrapure water	0
Sample injection volume	500 µL
NPOC purge time	180 s

## Results and Discussion

A defined measurement sequence was processed in parallel with two different device configurations. Samples, various control standards and nicotinic acid standards were measured alternately.

A total of 68 test vials were filled with 4 fillings per sample, system test and control standard. At least a triple injection was made from each vial.

The results are summarized in Table 2. The measurement order does not correspond to the presentation in the table but represents a summary of the control standards and samples measured across the sample sequence. The NPOC values of the samples were also recorded and are documented for informative reasons in brackets besides the sample names.

Table 2: Measurement results

Sample ID	Device configuration 1: multi N/C 3300 with CLD			Device configuration 2: multi N/C 3300 with ChD		
	Mean value TN <sub>b</sub> ± SD [mg/L]	RSD [%]	Recovery rate [%]	Mean value TN <sub>b</sub> ± SD [mg/L]	RSD [%]	Recovery rate [%]
Sample A NPOC: 5.30 mg/L	4.75 ± 0.17	3.6	-	4.83 ± 0.20	4.1	-
Sample B NPOC: 887 mg/L	23.6 ± 0.3	1.5	-	24.0 ± 0.3	1.3	-
Sample C NPOC: 156 mg/L	12.6 ± 0.3	2.4	-	12.7 ± 0.2	1.6	-
Sample D NPOC: 94.3 mg/L	36.8 ± 0.7	1.9	-	37.2 ± 0.6	1.6	-
Sample E NPOC: 47.8 mg/L	8.24 ± 0.12	1.5	-	8.19 ± 0.21	2.6	-
System test Nicotinic acid 5 mg/L N	5.08 ± 0.16	3.2	102	4.90 ± 0.02	0.4	98
System test Nicotinic acid 10 mg/L N	9.70 ± 0.16	1.6	97	10.1 ± 0.2	2.0	101
System test Nicotinic acid 20 mg/L N	20.7 ± 0.2	1.1	104	20.5 ± 0.1	0.5	102
System test Nicotinic acid 50 mg/L N	50.7 ± 0.5	1.0	101	49.2 ± 0.5	1.0	98
Control standard NO <sub>3</sub> + NH <sub>4</sub> (1:1), 5 mg/L N	5.01 ± 0.15	3.0	100	4.94 ± 0.06	1.3	99
Control standard NO <sub>3</sub> + NH <sub>4</sub> (1:1), 10 mg/L N	9.56 ± 0.04	0.4	96	10.1 ± 0.1	1.1	101
Control standard NO <sub>3</sub> + NH <sub>4</sub> (1:1), 20 mg/L N	20.2 ± 0.4	1.8	101	20.4 ± 0.2	0.9	102
Control standard NO <sub>3</sub> + NH <sub>4</sub> (1:1), 50 mg/L N	49.7 ± 1.2	2.5	99	49.9 ± 0.4	0.9	100
Control standard NO <sub>3</sub> 10 mg/L N	9.66 ± 0.25	2.6	97	10.2 ± 0.4	3.9	102
Control standard NO <sub>3</sub> 50 mg/L N	51.9 ± 1.1	2.1	104	51.9 ± 1.0	2.0	104
Control standard NH <sub>4</sub> 10 mg/L N	10.1 ± 0.4	3.8	101	9.91 ± 0.08	0.8	99
Control standard NH <sub>4</sub> 50 mg/L N	50.1 ± 1.3	2.6	100	50.0 ± 0.1	0.2	100

The following figures 3 to 6 show typical nitrogen measurement curves for selected examples.

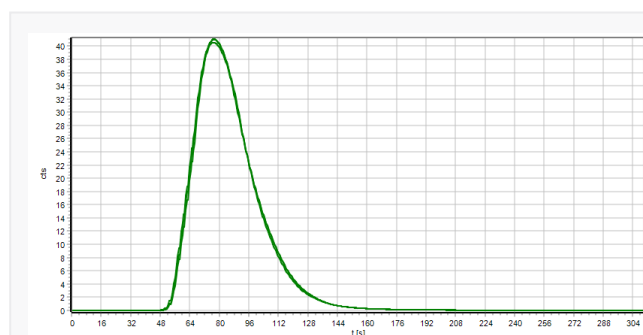


Fig. 3: TN<sub>b</sub> measuring curve sample A (surface water) with CLD

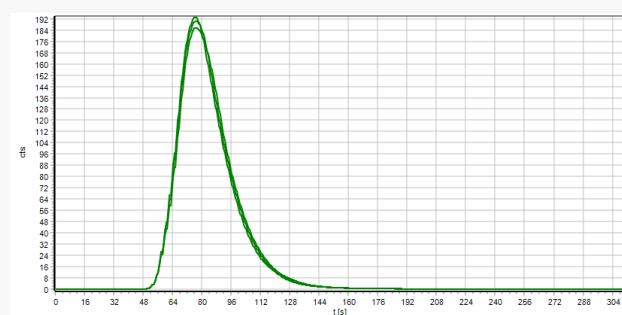


Fig. 4: TN<sub>b</sub> measuring curve sample B (process water) with ChD

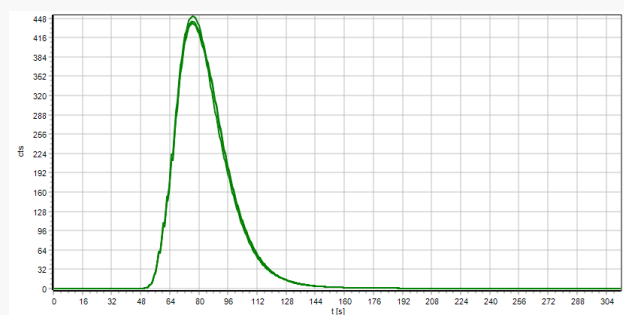


Fig. 5: TN<sub>b</sub> measuring curve nicotinic acid standard 50 mg/L N with CLD

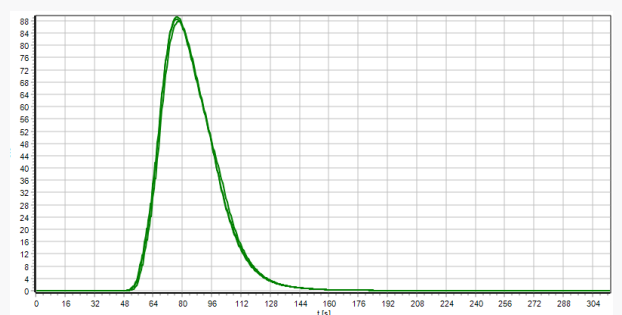


Fig. 6: TN<sub>b</sub> measuring curve KNO<sub>3</sub> standard 10 mg/L N with ChD

Looking in particular at the results for the nicotinic acid solutions with the nitrogen concentrations 5 mg/L, 10 mg/L, 20 mg/L, and 50 mg/L, which were measured as part of the system test, it becomes clear that the test is fulfilled without exception over the entire concentration range. On the one hand, the measured values fulfill the criterion that the deviation from the theoretical value must not exceed  $\pm 5\%$ . This is confirmed by the recovery rates, which are all in a range between 97 and 104%. On the other hand, the excellent reproducibility of the nitrogen measurement values of the system test solutions proves the stability of the analysis system. Standard deviations in the range of 0.02 to 0.5 mg/L (corresponding to 0.4% to 3.2% relative standard deviation) were achieved. These were calculated from at least 12 sample injections (individual measured values) of a standard, whereby the standards were distributed over the entire measurement sequence. This more than fulfills the requirements of DIN EN ISO 20236 with regard to the coefficient of variation for the nicotinic acid solutions. The deviation of the individual values in the range of < 10 mg/L N is also less than 1 mg/L for all system test samples.

Furthermore, highly reproducible sample results were achieved. Like the system test solutions, the samples were distributed in blocks over the entire measurement series and were also intended to expose the combustion tube to a certain matrix load. To check the calibration stability, control standards of potassium nitrate and ammonium sulphate

were also constantly analyzed in the series of measurements. Here too, the recovery rates in the range of 96% to 102% prove the reliability and stability of the analyzer and the calibration.

Furthermore, the TN<sub>b</sub> content was examined in control solutions that contained either only nitrate-nitrogen or only ammonium-nitrogen. Here, DIN EN ISO 20236 points out that calibration with a NO<sub>3</sub>/NH<sub>4</sub> mixed standard can lead to overestimation in the case of nitrate-N determinations and to underestimation in the case of ammonium-N determinations. The analyzed nitrate standard solutions with 10 and 50 mg/L N were determined with a very good recovery in the range between 97% and 104%. The same applies to the analyzed ammonium standards with 10 and 50 mg/L N. Here the recovery rates were 99% to 101%. This means that neither over- nor under-detections were recorded for the individual nitrate and ammonium standards, which underlines the high performance of the analytical systems used.

In summary, it can be stated that the measurement data generated with two different device configurations show no significant differences in the quality of the measured values in relation to the detection system used (CLD or ChD). Both detection methods fully comply with the requirements of DIN EN ISO 20236 for nitrogen determination.

## Summary

The systems of the multi N/C x300 series are characterized by excellent performance characteristics in the determination of total bound nitrogen in accordance with DIN EN ISO 20236. Two equivalent detection methods can be used for this purpose, either chemiluminescence or a maintenance-free electrochemical method, which is used in the chemodetector. In addition to the multi N/C 3300 basic device with its loop injection technology, an analyzer with direct injection technology, the multi N/C 2300, can also be used for this application. The multi N/C 2300 can also be coupled with one of the two detectors (CLD or ChD) and achieves comparable performance characteristics for nitrogen determination. To determine the total organic carbon in water of different origins in accordance with DIN EN ISO 20236, numerous tests have also been carried out with the systems of the multi N/C x300 series and the conformity with standard methods has been confirmed without restriction. The analyzers are characterized in particular by long-term stable calibration, effective particle handling, and low wear of consumables. Intuitive operation of the devices using modern software is a matter of course.



Figure 7: multi N/C 3300 with AS vario (left) and multi N/C 2300 with AS 60 (right)

The TOC/TN<sub>b</sub> analyzers of the multi N/C x300 series guarantee reliable and economical routine analysis for the determination of both parameters in water samples in accordance with DIN EN ISO 20236 at all times.

## Recommended device configuration

Table 3: Overview of devices, accessories, and consumables

Article	Article number	Description
multi N/C 3300 CLD	450-500.502	TOC/TN <sub>b</sub> analyzer with flow injection technology and chemiluminescence detector for N determination
multi N/C 3300 ChD	450-500.501	TOC/TN <sub>b</sub> analyzer with flow injection technology and chemodetector for N determination
AS vario	450-900.140	Autosampler for multi N/C 3300
Sample rack with 72 positions	450-900.141	Accessory for AS vario
multi N/C 2300 CLD	450-500.102	TOC/TN <sub>b</sub> analyzer with direct injection technology and chemiluminescence detector for N determination
multi N/C 2300 ChD	450-500.101	TOC/TN <sub>b</sub> analyzer with direct injection technology and chemodetector for N determination
AS 60	450-126.682	Autosampler for multi N/C 2300

## References

- [1] DIN EN ISO 20236:2023-04 „Water quality - Determination of total organic carbon (TOC), dissolved organic carbon (DOC), bound nitrogen (TN<sub>b</sub>) and dissolved bound nitrogen (DN<sub>b</sub>) after catalytic oxidative high-temperature combustion (ISO 20236:2018)“
- [2] DIN EN 12260:2003 “Water quality - Determination of nitrogen - Determination of bound nitrogen (TN<sub>b</sub>), following oxidation to nitrogen oxides”

This document is true and correct at the time of publication; the information within is subject to change. Other documents may supersede this document, including technical modifications and corrections.

Trademark notice: The brand names of the third-party products specified in the application note are usually registered trademarks of the respective companies or organizations.

## Headquarters

Analytik Jena GmbH+Co. KG  
 Konrad-Zuse-Strasse 1  
 07745 Jena · Germany

Phone +49 3641 77 70  
 Fax +49 3641 77 9279

info@analytik-jena.com  
 www.analytik-jena.com

Version 1.0 · Author: BW  
 en · 07/2024  
 © Analytik Jena GmbH+Co. KG  
 Picture p. 1 ©: Adobe Stock/Space Priest