Challenge

TN samples from cleaning validation can have strongly varying concentrations, which require an analyzer that is free of carryover and has a high detection capacity.

Solution

The performance of the high temperature analyzers of the multi N/C x300 series for use in cleaning validation is demonstrated using series of measurements with samples of different concentration levels.

Intended audience

Pharmaceutical and biopharmaceutical industry, contract laboratories for pharmaceutical analysis.

GMP-Compliant TN Cleaning Validation in the Biopharmaceutical Industry

Introduction

In contrast to traditional pharmaceutical production, which uses chemical reactions to produce active pharmaceutical ingredients (API), biopharmaceutical production processes often use genetically modified organisms (bacteria, fungi, etc.) to produce pharmacologic substances or extract drug substances from plant or animal materials. These active ingredients are usually polypeptides or proteins composed of various amino acids. In all bio-pharmaceutically produced APIs and pharmaceuticals, nitrogen is present in addition to carbon. For this reason, the parameter TN (Total Nitrogen), which is significantly more active substance-specific than the parameter TOC (Total Organic Carbon), is ideally suited for use as a key parameter for cleaning validation in biopharmacy.

In addition to the widely used TOC determination as a non-specific analysis method for organic impurities in cleaning validation, the determination of TN is particularly useful because the risk of contamination of samples by external influences is significantly lower than with TOC. Samples obtained from the post-final rinse process or swab extraction often contain very low concentrations of organic compounds which, in the case of carbon determination, can be quickly contaminated and thus falsified by contamination from the ambient air or the sampling vessels themselves. This applies to nitrogen determination in very rare cases only and to a much lesser extent.

Methods for instrumental total nitrogen analysis are described in various pharmacopoeias (EP 2.5.33, method 7B[1]; USP <1057>, method 7.2[2]; JP XVII, method 7B[3]) and are therefore generally acknowledged. The method for determining TN is based on a catalytically assisted high-temperature oxidation of the sample at high temperatures of up to 1000 °C in an oxygen-rich atmosphere. The subsequent detection of the NO formed in the process is

carried out using a chemiluminescence detector (CLD). Instrumental nitrogen analyzers generally enable the parallel determination of TOC, which can be an advantage for biopharmaceutical laboratories if organic carbon impurities also need to be analyzed in the water used for production. TN determination provides a simple and effective analytical measurement method for verifying the effectiveness of cleaning processes used in production facilities in accordance with the GMP guideline ICH Q71.

This paper shows how TN analyzers of the multi N/C x300 series not only meet the requirements of the pharmacopoeias with regard to nitrogen determination in cleaning validation but also impress with very good performance characteristics in terms of detection strength and carry-over-free analysis with changing nitrogen concentrations.

Materials and Methods

The TN determinations were carried out on the multi N/C 3300 and multi N/C 3300 HS analyzers. The samples and standards were injected directly into the catalyst-filled combustion tube of the analyzer without any additional sample preparation. The nitrogen compounds contained in the sample are completely oxidized there at high temperatures. The nitrogen oxides formed in this process are then passed through a chemiluminescence detector (CLD) for quantification. The AS vario autosampler was used for automatic sample feeding in combination with a tray for 72 samples of 40 ml each.
The two measuring devices differ only in their sample injection technique. Both devices are dosing the liquid sample via a syringe pump. On the multi N/C 3300, however, the sample is drawn into a sample loop, which prevents contamination of the syringe pump with the sample. By reverse rinse of the sample loop and utilization of large tube diameters, even samples with a challenging matrix (e.g. high particle load) can be analyzed without carryover using flow injection principle. The multi N/C 3300 HS is optimized for ultrapure water analysis. The sample is drawn directly into the syringe pump, allowing higher sample volumes to be injected (up to 3 mL) and lower detection limits to be achieved.

Samples and reagents

- Nitrogen calibration solutions in the concentration range 0.1 mg/L to 10 mg/L, produced from bovine serum albumin (BSA) (Sigma-Aldrich Art. No. A-7906, Albumin, Bovine, with an N content of 15.60% and a purity of 98%)
- BSA reference solutions from Reagecon for the system test, concentrations 1 mg/L and 10 mg/L N
- Control standard solutions from BSA, concentrations 0.5 mg/L and 10 mg/L
- 2 samples (containing organo-nitrogen compounds) from the cleaning validation (A, B)

Sample preparation

All samples and standards were stored in the refrigerator at approx. 4 °C until they were measured. After appropriate warming to room temperature, the samples were filled into 40 mL sample vials, covered with aluminum foil and placed on the tray of the autosampler.
All calibration and control standard solutions were freshly prepared from their corresponding stock solutions on the day of measurement.

Calibration

A multi-point calibration in the concentration range from 0.1 mg/L to 10 mg/L N was carried out for both analyzers. BSA calibration solutions were used for this to ensure calibration as close to the matrix as possible. The solutions were prepared from a BSA stock solution with 100 mg/L. For the stock solution, 64.1 mg BSA was dissolved in 100 mL ultrapure water. Calibration with a TN mixed standard (equal parts N from KNO_3 and $(NH_4)_2SO_4$) would also be possible at this point.
The calibration curves were evaluated using linear regression. The calibration range was divided into two sections to ensure the highest possible accuracy at each calibration point.

The calibration parameters using the multi N/C 3300 are listed in Table 1 and the calibration curve is shown in Figure 1.

Table 1: Calibration parameter

Range	k0	k1	R^2	Limit of detection [mg/L]	Limit of quantification [mg/L]
1	-0.64	960.46	0.99992	0.02	0.07
2	-105.07	890.02	0.99965	0.10	0.37

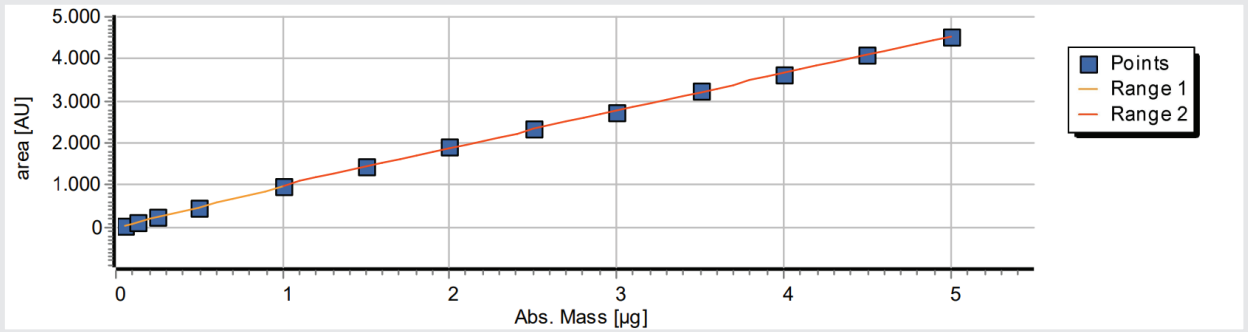

Figure 1: Calibration curve with two calibration ranges from 0.1 to 0.5 mg/L and 0.5 to 10 mg/L TN

Instrument and method settings

The device and method parameters for both instruments are listed in Table 2.

Table 2: Device and method settings for standard and sample measurements

Parameter	multi N/C 3300	multi N/C 3300 HS
Method parameter	TN	
Digestion	High-temperature combustion with platinum catalyst	
Combustion temperature	750 °C	
Carrier gas	Synthetic air	
Number of repeat measurements per vessel	min. 3, max. 4	
Autosampler, tray and vessel sizes	AS vario, rack with 72 positions, 40 mL sample vessels	
Number of rinsing cycles with sample before the 1st injection	3	
Injection volume	500 µL	1000 µL

Results and Discussion

A defined measurement sequence was carried out in parallel on both measuring devices. For this purpose, the samples, various control standards and ultrapure water samples were measured alternately.
The series of measurements consisted of 29 sample vials filled with either samples or standards; at least one triple injection was made from each vial.
The results are summarized in Table 3. The measurement sequence does not correspond to the presentation in the table but represents a summary of the control standards and samples measured across the sample rack. In the measurement sequence, the control standards were measured first, followed by the samples. An ultrapure water determination was then carried out to check for possible carryover. Finally, the standards for the system test were analyzed.

Table 3: Results of TN determination

Sample ID	TN nominal concentration [mg/L]	Number of vials, 3 to 4 injections each	TN measured concentration mean value [mg/L]	RSD [%]	Recovery [%]
Sample A	-	5	2.97	0.46	-
Sample B	-	5	0.57	1.40	-
Control standard BSA, Sigma-Aldrich	0.5	5	0.48	0.83	95.7
Control standard BSA, Sigma-Aldrich	10.0	5	10.32	0.49	103.2
System test BSA, Reagecon	1.0	3	0.98	2.28	97.8
System test BSA, Reagecon	10.0	3	10.37	0.39	103.7
Ultrapure Water	-	5	< LOD	-	-

Figures 2 to 5 below show typical nitrogen measurement curves based on selected examples.

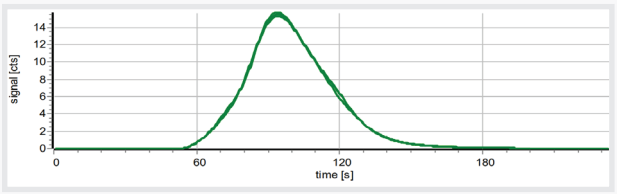

Figure 2: TN measurement curve Sample A

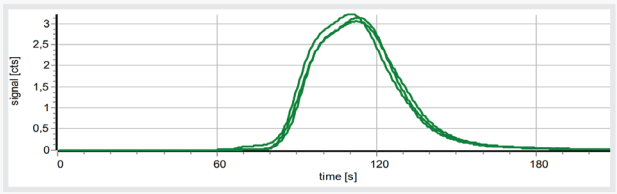

Figure 3: TN measurement curve Sample B

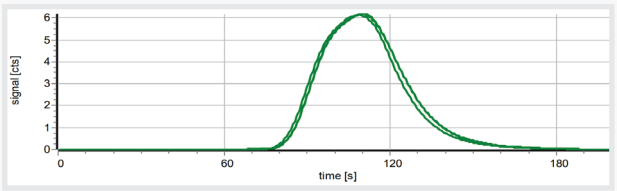

Figure 4: TN measurement curve System Test 1 mg/L

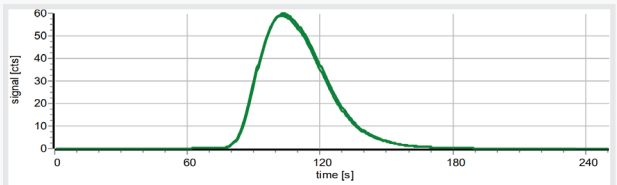

Figure 5: TN measurement curve Control Standard 10 mg/L

The analysis of the ultrapure water samples on both measuring devices showed that the samples did not lead to any carryover of TN compounds.
Looking in particular at the results for Reagecon's BSA solutions with nitrogen concentrations of 1 mg/L and 10 mg/L, which were measured as part of the system test, it is clear that both calibration ranges are valid. On the one hand, the measured values show a deviation of less than ± 5% from the theoretical value. The recovery is 98% and 104% respectively. On the other hand, the excellent reproducibility of the nitrogen measurement values of the system test solutions proves the stability of the analysis system. Relative standard deviations of 2.3% and 0.4% were achieved. These were calculated from at least 9 sample injections (individual measured values) of a standard, whereby the standards were distributed over the entire measurement sequence.

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 made of BSA were constantly analyzed in the series of measurements. Here too, the recovery rates of 97% and 103% demonstrate the reliability and stability of the analyzer and calibration respectively.
In conclusion, it can be stated that no significant differences in the quality of the measured values can be derived from the measurement data generated with two different devices. Both measuring devices fully meet the requirements of EP 2.5.33, method 7B; USP <1057>, method 7.2 and JP XVIII <G3-12-172> method 7B for nitrogen determination.

Summary

The systems of the multi N/C x300 series are characterized by excellent performance characteristics in the determination of nitrogen compounds for the purpose of cleaning validation (EP 2.5.33, method 7B; USP <1057>, method 7.2; JP XVIII <G3-12-172>, method 7B). Two equivalent measuring devices can be used for this purpose, the multi N/C 3300 and the multi N/C 3300 HS. Both devices achieve low detection limits (<0.05 mg/L) and provide consistent measurement results for both the samples and the control standards.
Intuitive operation of the devices using modern software is a matter of course.
In addition to TN cleaning validation, the two TOC/TN analyzers of the multi N/C x300 series can also be used to carry out measurements for ultrapure water monitoring for organic carbon contamination (TOC) or for TOC cleaning validation in accordance with EP 2.2.44., USP <643> and JP 2.59.

Figure 6: multi N/C 3300 with AS vario

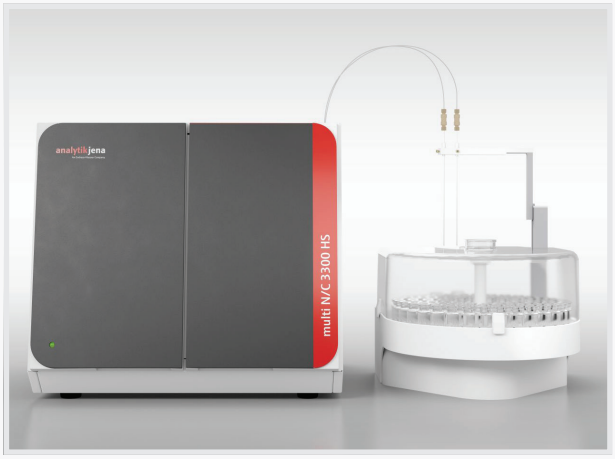

Figure 7: multi N/C 3300 HS with AS vario

Recommended device configuration

Table 4: Overview of devices, accessories, and consumables

Article	Article number	Description
multi N/C 3300 CLD	450-500.502	TOC/TN_b analyzer with flow injection technology and chemiluminescence detector for N determination
multi N/C 3300 HS	450-500.600	TOC/TN_b analyzer with flow injection technology and upgradeable chemiluminescence detector for N determination
CLD-300	450-500.650	Upgradeable chemiluminescence detector for N determination
AS vario	450-900.140	Autosampler for multi N/C x300
Rack with 72 positions	450-900.141	Accessory for AS vario

References

[1] EP 2.5.33 Total Protein, Method 7, Procedure B
[2] USP <1057> BIOTECHNOLOGY-DERIVED ARTICLES—TOTAL PROTEIN ASSAY, Method 7, Procedure 2
[3] JP XVIII <G3-12-172> Total Protein Assay, Method 7, Procedure B

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: TK
en · 03/2025