Challenge
Total protein analysis from small sample volumes in pharmaceutical products

Solution
Fast, safe, and reproducible analysis with a high level of automation and sample throughput using the multi N/C 2300 N

Total Protein Assay in Bio-Pharmaceutical Products (e.g. Vaccines) by TN Analysis via Catalytic Combustion

Introduction

In pharmaceutical vaccine production, starting, intermediate, and end products need to be controlled for the level of antigens. They are tested for the quantity of attenuated or devitalized viruses or bacteria. Since these antigens typically consist of proteins, the analytical quantification of total protein is a method of choice. Pharmacopoeia regulations list a number of relevant methods, among them are several UV/Vis assays (e.g., Lowry, Bradford, BCA assay) and two total nitrogen methods, which is the Kjeldahl and the catalytic combustion method.

This application note focuses on the catalytic combustion method described in the European Pharmacopoeia Monograph 2.5.33, Method 7 B[1], also in USP <1051, 7.2> [2] and JP XVIII <G3-12-172>[3]: high temperature pyrolysis of the nitrogen compounds in an oxygen atmosphere to nitric oxide (NO) followed by chemiluminescence detection (CLD). This method description for total nitrogen (TN) determination almost coincides with the DIN EN ISO 20236[4], which describes the determination of total bound nitrogen (TN_b) in environmental water samples by catalytic high temperature combustion and CLD detection of the formed NO molecules. The multi N/C x300 analyzers can be applied for TN determination according the listed pharmacopoeia methods in bio-pharmaceutical products like vaccines. The TN can be converted into total protein content to determine the level of antigens. This calculation of total protein results from TN readings can be performed inside the multiWin pro software fully automatically.
Since the nitrogen content of proteins varies, it is widely accepted to convert total nitrogen concentrations into total protein concentrations by multiplying a factor of 6.25 according to the following formula:

$$C\,[\text{Total Protein}] = c\,[\text{total nitrogen}] \times 6.25$$

Materials and Methods

Samples and reagents

Five urea TN calibration standards prepared at a customer site, one BSA control standard, and three unknown customer samples were measured in triplicate determination. The samples were stored at 4 °C in the refrigerator until analysis.

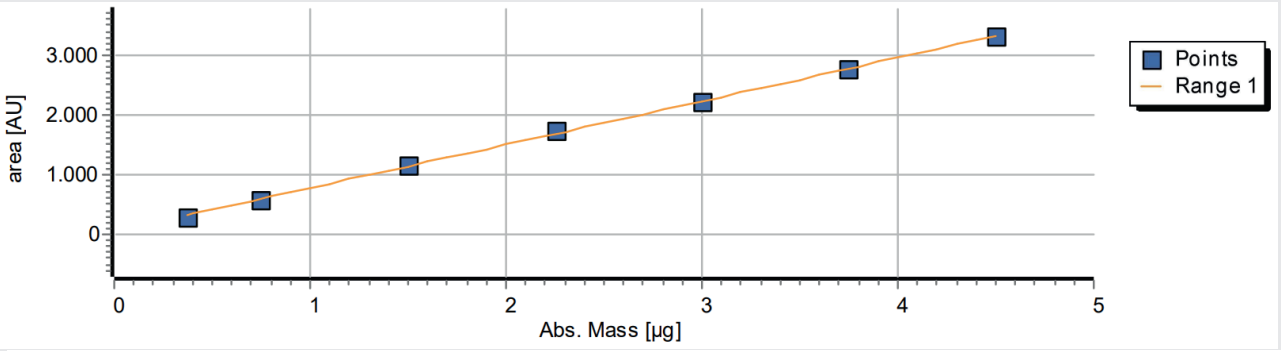
After conditioning at room temperature the liquid samples were directly transferred into 2 mL sample vials using a micro pipette and covered with snap-caps. 75 µL of sample aliquots were transferred into the furnace of the analyzer by aid of the microliter syringe of the autosampler. Supported by platinum catalyst all nitrogen compounds were converted to nitrogen monoxide (NO) in a pure oxygen atmosphere using catalytic high-temperature combustion. The NO was subsequently detected quantitatively by means of a chemiluminescence detector. The measurement sequence was supported by the autosampler AS 60 with automatic magnetic stirring of the sampling position and syringe wash station.

Calibration

The multi N/C 2300 N was calibrated from 5 to 60 mg/L for total nitrogen (TN) with a multi-point calibration using a BSA protein standard solution. A BSA stock solution of 200 mg TN/L was prepared (Sigma Art.Nr. A-7906, Albumin, Bovine with N-content of 15.60 % and purity grade of 98 %) weighing 128.2 mg for 100 mL ultra-pure water.
The standard conversion factor for such kind of samples (found in literature) for calculating the protein concentration from the measured N concentration of 6.25 was used to calculate the protein concentration of the measured samples. The calibration curves can be found in Figure 1.

Instrumentation

The analysis was performed on the multi N/C 2300 N. The method settings shown in Table 1 were used to determine the TOC content.



#	k0	k1	k2	DF	R^2	Limit of Detection	Limit of Quantification	Range borders
0	38,99	729,63	-	-	0,99940	0,97 mg/L	3,56 mg/L	0,375 µg to 4,500 µg

Figure 1 a-b: Example of TN – BSA Calibration curve and characteristics range 5–60 mg/L N

Table 1: Method settings

Parameter	multi N/C 2300 N
Measurement parameters	TN
Digestion	High temperature digestion at 800 °C with platinum catalyst
Number of single repetitions	min. 3, max. 4
Rinse with sample before injection	3 times
Injection volume	75 µL
Dilution	1:1

Results and Discussion

Each sample has been measured at least three times. The results are listed in the following table and measuring curves.

Table 2: Liquid samples

Sample ID	Mean value TN [µg/mL] ± RSD [%] (1. vial)	Mean value TN [µgm/L] ± RSD [%] (2. vial)	Mean value TN [µg/mL] ± RSD [%] (3. vial)
10 ppm Nitrogen Std 10.77 ± 0.14	10.77 ± 0.14	10.80 ± 0.51	10.74 ± 0.19
20 ppm Nitrogen Std 20.02 ± 0.20	20.02 ± 0.20	20.05 ± 0.27	19.98 ± 0.24
30 ppm Nitrogen Std 30.31 ± 0.43	30.31 ± 0.43	30.35 ± 0.28	30.42 ± 0.30
40 ppm Nitrogen Std 40.59 ±	40.59 ± 0.27	40.62 ± 0.42	40.65 ± 0.35
50 ppm Nitrogen Std 50.74 ± 0.11	50.74 ± 0.11	50.96 ± 0.13	50.95 ± 0.29
30 ppm BSA checking Std 30.80 ±	30.80 ± 0.54	30.89 ± 0.45	30.88 ± 0.40
Sample A	17.22 ± 0.34	17.20 ± 0.52	17.18 ± 0.44
Sample B	27.95 ± 0.22	27.97 ± 0.21	27.93 ± 0.40
Sample C	48.27 ± 0.34	48.25 ± 0.17	48.05 ± 0.09

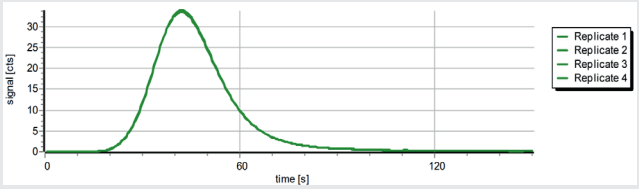


Figure 2a: Example 30 ppm BSA checking Std

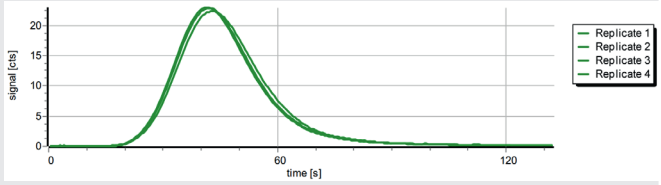


Figure 2b: Sample A

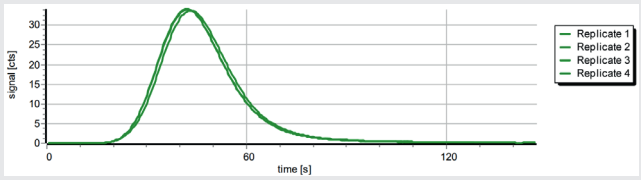


Figure 2c: Sample B

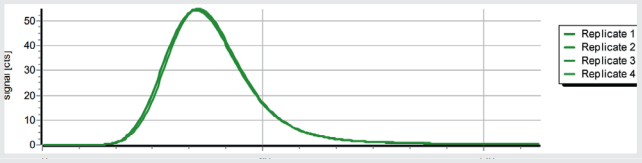


Figure 2d: Sample C

Summary

All samples were measured with very good repeatability and low RSDs. The clear advantage of a direct injection system using a microliter syringe is the short and direct sample transfer from the vial to the combustion furnace without long tubings and valves. This allows optimized small rinse volumes of max. three times 75 µL. Sample consumption can be kept very low. For five replicates including three rinse cycles and a representative injection volume of 75 µL less than 1.5 mL of sample is required.

multi N/C 2300 N equipped with a CLD detector for total nitrogen determination is the optimum system to perform total protein assay for such vaccine samples for such samples according to the catalytic high temperature digestion method described in EP, USP and JP.

The recovery of the customer calibration standard solutions and checking standard (BSA), as well as the presented peak shapes of the sample runs, which show no shifts between replicates nor significant peak tailing, proof the performance of the analyzer.

Figure 3: multi N/C 2300 N with autosampler AS 60

References

[1] I 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

[4] DIN EN ISO 20236, Water quality – Determination of total organic carbon (TOC), dissolved organic carbon (DOC), total bound nitrogen (TNb) and dissolved bound nitrogen (DNb) after high temperature catalytic oxidative combustion

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