solutions

TOC in pharma

Cleaning Validation

TOC Performance Test

Extractables Testing

Total Protein Determination

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Dear readers,

TOC (total organic carbon) is a parameter of growing importance in the production and packaging of pharmaceuticals. The TOC content can be used to draw conclusions about organic impurities. The determination of TOC in order to verify a defined quality and purity in water is therefore indispensable in many pharmaceutical applications. In this issue of solutions, we highlight some of the most common pharmaceutical TOC and TN applications: Ultrapure water testing and cleaning validation, analysis of extractable organic components from packaging materials and quality control of vaccines.
We present simple, effective methods to address these routine challenges. Two things are particularly important to us. On the one hand, we would like to offer you a guidebook that supports you in your daily laboratory routine and brings you closer to reliable methods and instrument options. On the other hand, we like to show you ways in which you can design these applications economically at the same time. After all, these are mostly routine applications that have to be performed frequently and with large sample quantities in pharmaceutical laboratories. Factors such as sample throughput, reliability of results, maintenance and downtime or service offerings play a major role.
We wish you many valuable insights and will of course be happy to advise you.

Yours sincerely

Bernd Bletzinger, Product Manager TOC

Content

Cleaning Validation Procedures in the Pharmaceutical Industry by TOC Analysis

Introduction

To minimize or prevent cross contamination from product to product in pharmaceutical production equipment, manufacturers are obliged to establish defined cleaning processes in accordance with the pharmaceutical operating regulations. According to the ICH Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, the effectiveness of these cleaning procedures is to be demonstrated regularly by the use of analytical measuring techniques. This means that after successful cleaning a check must be carried out for residues of active pharmaceutical ingredients (API), additives, detergents and their decomposition and reaction products, using a representative and validated sampling and analysis method.
Both, substance-specific techniques (HPLC, GC, etc.), as well as non-specific analysis techniques (sum parameters TOC or TN) are used, other indicators are conductivity, pH and surface tension. Since each of the possible contaminants listed above typically represents organic compounds and can be addressed by total organic carbon, TOC has been chosen and pushed by the FDA to become the number one non-substance specific screening parameter in cleaning validation.

Challenge

TOC samples derived from cleaning validation are characterized by changing concentration levels, demanding a TOC analyzer without memory or carry-over effects.

Solution

Successful demonstration of a measurement sequence of samples with trace level and higher TOC concentrations and calibration strategy to cover a wide linear TOC working range.

Additionally TOC determination is a mandatory parameter in WFI (water for injection) and AP (aqua purificata – purified water for pharmaceutical use) quality control and a well described pharmacopoeia method with ultralow detection limits below 50 ppb according to Pharm. Eur. 2.2.44 and USP <643> monographs.
Cleaning validation limits and acceptance criteria are calculated according to different approaches listed in the PDA technical report no. 29 and 49, e.g., based on drug active dose or toxicity to establish acceptable residue levels (ARL).
Two strategies – the post-final rinse and the swab test – are followed during cleaning validation to prove the cleanliness of production equipment. The particular advantage of the post final rinse or swab extracts procedure is that both sampling approaches can be established more easily, are less error-influenced and the resulting TOC samples can be processed by a standard TOC analyzer using typical method settings and quality assurance checks.

Materials and methods

Samples and reagents
Two customer-provided rinse samples and two samples from swab surface sampling were prepared and analyzed according to USP resp. Pharm. Eur. guidelines for Total Organic Carbon measurements.

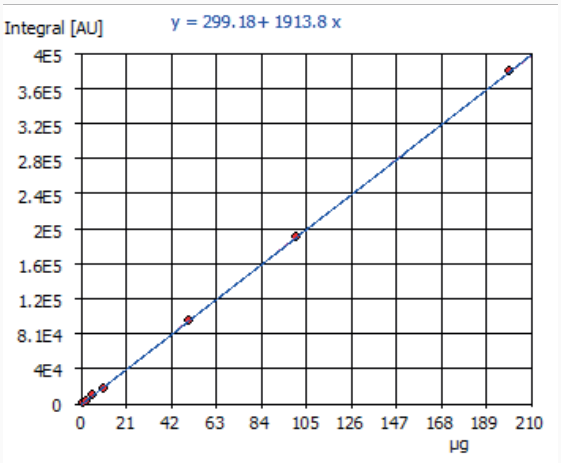
Sample preparation and measurement
In the post-final rinse the production equipment is rinsed once more after the final rinse of the cleaning procedure to transfer any potential organic surface contamination into this rinse water and to make it available for TOC measurement.

With the swab test (Figure 1a, 1b), on the other hand, previously defined risk locations, such as recesses, welding seams and obstacles, are purposefully sampled by using e.g., cotton or polymer fiber swabs. The swab material is moistened and rinsed with ultrapure water before and during the sampling. The sampling area, usually limited using a template (normally approx. 100 cm^2) is wiped in layers cross-wise. The swab is then eluted/extracted with ultrapure water, by aid of shaking or sonication, topped up to a fixed volume of for example 40 mL and subsequently measured for TOC content. A special procedure is the swab sampling for completely water-insoluble contaminants using inorganic fiber swabs (e.g., quartz fleece) to wipe the equipment surfaces for mechanical sampling. Subsequent direct swab combustion by catalytic high-temperature oxidation is applied for determination of the TOC load on the swab material. However, in this procedure various factors must be taken into account, such as the availability of swab materials with a consistently low TOC blank value, loss of fiber material during sampling or even surface abrasion by the wiping process.
The rinse samples were collected during the post-final rinse process with pure water. The swab samples were provided readily extracted with pure water in 40 mL vials. Sample vials were directly placed onto the autosampler without transferring the sample into other vials. Automatic acidification was performed to a pH <2 and as part of NPOC sample preparation the TIC was purged from the acidified samples automatically by a carrier gas stream. Further method parameters are referenced in the instrumentation section below. The formed CO_2 gas was transferred by a carrier gas stream into the Focus Radiation NDIR detector for quantification.

Figure 1a, 1b: Swab sampling on a test specimen, cross-wise in lanes

Calibration

The analyzers of the multi N/C pharma series were calibrated for NPOC in the range from 0.1 to 20 mg/L with standard solutions prepared from a sucrose stock solution containing 100 mg/L C. A multi-point calibration type was used. The calibration curve and its characteristics are presented in Figure 2.
An outstanding linearity could be demonstrated throughout the whole calibration range from 0.1 to 20 mg/L for all three TOC analyzer models of the multi N/C pharma series.



Residual SD:	353.06AU	Linearity:	OK
Method SD:	18.45µg/l	Variance homogeneity:	OK
Method VC:	0.35091%	Detection limit:	27.80µg/l
Qual. of rep.:	0.99999	Identification limit:	55.60µg/l
Correl. coeff.:	1	Quantification limit:	106.2µg/l

Figure 2: NPOC – calibration curve and characteristics, performed by multi N/C pharma UV.

Instrumentation

TOC measurements were performed on all pharma TOC analyzers: the multi N/C pharma UV, the multi N/C pharma HT and the multi N/C 3100 pharma. Following method settings were used to determine the TOC content (Table 1):

Table 1: Method settings

	multi N/C pharma UV	multi N/C pharma HT, multi N/C 3100 pharma
Parameter	NPOC (direct TOC measurement)	NPOC (direct TOC measurement)
Digestion	UV radiation assisted by $Na_2S_2O_8$	High-temperature oxidation using Pt catalyst at 800 °C
Number of repetitions	Min. 3, max. 4	Min. 3, max. 4
NPOC purge time	300 s	300 s
Rinse with sample before injection	3 times	3 times
Injection volume	5 mL	2 mL (pharma HT), 1 mL (3100 pharma)

Results

Four cleaning validation samples were measured alongside with different QC check standards and pure water samples in one sequence after system calibration as described above. Results for multi N/C 3100 pharma are summarized in Table 2 and Figure 3.

Table 2: Results

Sample ID	NPOC Average [mg/L]	RSD [%]
Post final rinse sample 1	0.327	1.9
Post final rinse sample 2	0.943	1.3
QC sample 1 (0.5 mg/L NPOC)	0.509	1.6
QC sample 2 (20 mg/L NPOC)	20.11	0.6
Pure water sample	0.058	3.7
Swab extractsample 1	1.742	0.9
Swab extractsample 2	15.79	0.5
QC sample 1 (0.5 mg/L NPOC)	0.504	1.7
QC sample 2 (20 mg/L NPOC)	20.22	0.7
Pure water sample	0.065	3.3

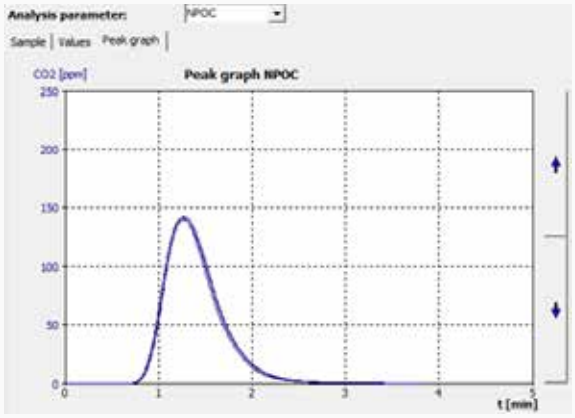


Figure 3: TOC peak graph for extracted swab sample 1

Conclusion

The results clearly demonstrate that the TOC analyzers of the multi N/C pharma series provide very good performance characteristics for the measurement of cleaning validation samples. Very low TOC concentrations can be determined besides higher loaded samples with high precision and accuracy. The instruments do not show carry-over effects in case of higher polluted samples which might occur in a sample sequence. With their high oxidation power, the FR-NDIR detector and a sophisticated design the multi N/C pharma instruments allow reliable TOC determination in a wide linear measuring range.

With TOC analyzers from the multi N/C pharma series you are well prepared for the challenges of cleaning validation and pharmaceutical TOC testing.

multi N/C pharma

TOC & TN_b Analyzer

Excellence in Pharmaceutical TOC Testing

multi N/C pharma series

The multi N/C pharma series offers tailor-made solutions for this pharma-specific TOC analysis - ideal for ultrapure water control, cleaning validation and testing of extractable components from packaging materials.

- Reliable measurement of TC, TIC, TOC, NPOC, TN_b and more
- Long-term stable calibration
- Compliant to international pharmacopoeias
- High operational safety

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Challenge

After revision of USP TOC monographs, a wider concentration range has to be covered by the TOC method applied and an SST test at 8 ppm must be passed.

Solution

Adapted calibration strategy to cover a TOC working range up to 20 ppm and successful application of the state-of-the art SST test sequence in the multiWin software.

TOC System Suitability Test – Redefined According to New USP Regulations

Introduction

The USP <643> represents the general method for TOC testing in pharmaceutical applications and provides guidance on how to qualify the analytical technique for use as well as guidance on how to interpret instrument results for use as a limit test. The revised TOC monograph <643> was implemented with the release of USP 37. It now describes two different TOC testing approaches addressing different pure water qualities, which are "Bulk Water" (e.g., Purified Water [PF], Water for Injection [WFI], Water for Hemodialysis, and condensate of Pure Steam) and "Sterile Water" (e.g., Sterile Water for Injection [SWFI], Sterile Purified Water [SPW], Sterile Water for Irrigation, and Sterile Water for Inhalation).

For bulk water quality, the testing procedure and limit values were retained as established:

Table 3: Parameter and values for bulk water

Parameter	Values
Detection limit for the applied TOC analyzer	0.05 mg/L (ppm)
TOC limit for sample testing	0.5 mg/L
SST concentration level	0.5 mg/L
Max. preparation water TOC blank	0.1 mg/L

For the sterile water quality, the testing procedure was slightly modified and new limit values were established as follows:

Table 4: Parameter and values for sterile water

Parameter	Values
Detection limit for the applied TOC analyzer	0.1 mg/L (ppm)
TOC limit for sample testing	8.0 mg/L
SST concentration level	8.0 mg/L
Max. preparation water TOC blank	0.5 mg/L

The following text demonstrates that the multi NC pharma allows straightforward and reliable TOC testing for sterile water according to the newly established SST.

Materials and methods

Sample preparation and measurement

Samples were taken from tap water and water from a filtration plant, where different filters were applied to clean up raw water. The samples had been stored in the refrigerator at 4 °C. System suitability test solutions of 8 mg/L sucrose and p-benzoquinone, respectively, were prepared from 100 mg/L stock solutions by dilution with ultrapure water from the ultrapure water plant in the lab and subsequently run with the SST sequence integrated in the software before the samples were measured.
After several rinse steps the samples were filled into 40 mL sampler vials, sealed with aluminum foil, and placed into the AS vario sample rack. Using the autosampler, the samples were acidified with 1 M H_2SO_4 (multi N/C pharma UV) and 2 M HCl (multi N/C 3100 pharma), respectively, and subsequently purged with the carrier gas according to the method settings for complete TIC removal prior to NPOC measurement.
During the oxidation process in the high-power, long-life UV reactor or in the Pt catalyst filled combustion tube, respectively all carbon compounds are quantitatively converted to CO_2. The wide-range Focus Radiation NDIR Detector was used for quantitative determination of CO_2 content in the measurement gas.

Calibration

The multi N/C pharma analyzers were calibrated for NPOC in the range from 0.2 to 20 mg/L with standard solutions prepared from a 1000 mg/L sucrose stock solution. A multipoint calibration type was used. The calibration curve and its characteristics are shown in Figure 4a and Figure 4b.

Residual SD:	143,47AU	Linearity:	OK
Method SD:	15,97µg/l	Variance homogeneity:	OK
Method VC:	1,5207%	Detection limit:	45,10µg/l
Qual. of rep.:	0,99984	Identification limit:	90,19µg/l
Correl. coeff.:	0,99992	Quantification limit:	190,1µg/l

Figure 4a: NPOC characteristics

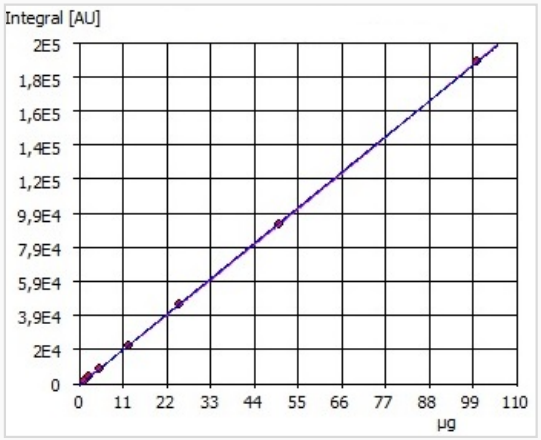


Figure 4b: NPOC calibration curve

Instrumentation

The following method settings were used to determine the TOC content:

Table 5: Method settings

Parameter	multi N/C pharma UV	multi N/C pharma HT, multi N/C 3100 pharma
Measurement parameters	NPOC	NPOC
Digestion	UV radiation assisted by $Na_2S_2O_8$	High temperature oxidation using Pt catalyst at 800 °C
Number of single repetitions	Min. 3, max. 4	Min. 3, max. 4
NPOC purge time	360 sec	360 sec
Rinse with sample before injection	3 times	3 times
Injektion volume	5 mL	2 mL (pharma HT), 1 mL (3100 pharma)

Results and discussion

After system calibration, two tap water and two filtrated water samples were measured as described above and subsequent SST sequence run. Results are displayed in the table and figures below.

Table 6: Results

Sample ID	NPOC Average [mg/L]	NPOC RSD [%]
Tap water 1	0.856	1.1
Tap water 2	1.231	0.8
Filter 1	4.638	0.5
Filter 2	9.893	0.7

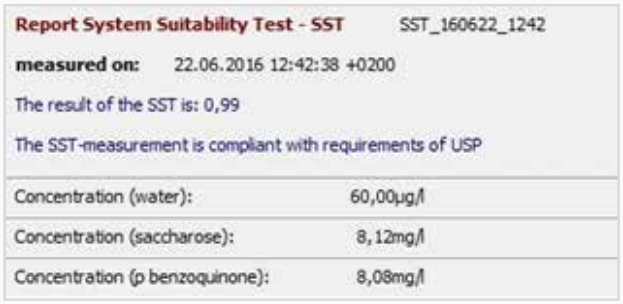


Figure 5: multiWin SST report

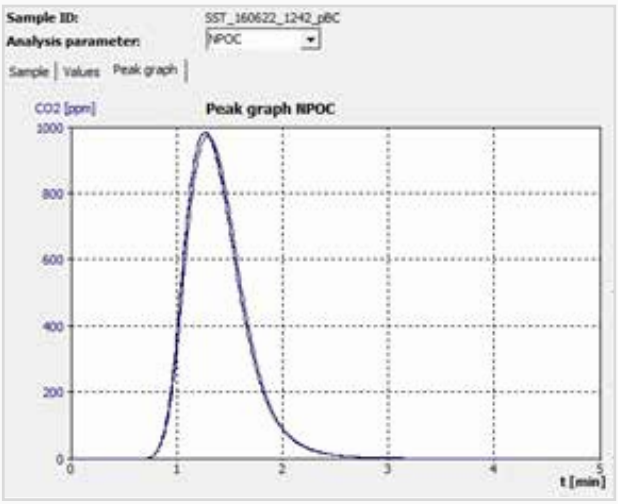


Figure 6: TOC peak graph for p-benzoquinone SST solution

Conclusion

The revision of the TOC-related USP monograph has broadened the pharma application range for TOC analyzers. Additionally, a new monograph USP <661.1> was implemented for testing of extractables from packing materials. According to this method, purified water extractions from polymer packing materials are prepared under the described conditions and tested for TOC within 4 hours after preparation according to USP <643>. The TOC method to be used needs to provide a linear dynamic range from 0.2 to 20 mg/L TOC with a detection limit of max. 0.2 mg/L.

This application note clearly demonstrates that the multi N/C pharma analyzers with their high-oxidation power and sophisticated design provide the required performance characteristics for the new challenges in pharmaceutical TOC testing beyond the 500 ppb limit.

Challenge

According to USP 661.1 and 661.2 TOC methods need to demonstrate a limit of detection of 0.2 mg/L and a linear dynamic range of 0.2 mg/L – 20 mg/L.

Solution

A calibration strategy to cover a TOC working range from 0.1 mg/L – 20 mg/L and successful demonstration of an equivalent linear dynamic range.

Establishing Extractables Testing from Plastic Packaging Materials and Systems for Pharmaceutical Use by TOC Analysis According to USP <661>

Introduction

The USP chapter <661> (formerly known as "Container-Plastics") was revised under the title "Plastic Packaging Systems and their Materials of Construction". The two new sub chapters of the new monograph describe TOC testing approaches of water-based extracts addressing "Plastic Materials of Construction" <USP 661.1> and "Plastic Packaging Systems for Pharmaceutical Use" <USP 661.2>. The whole chapter aims at further improving product safety of pharmaceutical products by using only well-characterized materials for packaging. Besides TOC testing these materials and systems should be characterized with regard to their identity, biocompatibility (biological reactivity), physicochemical properties (like UV/Vis absorbance, alkalinity or acidity), plastic additives and extractable metals.

According to USP <661.1> and <661.2> the TOC methods used need to provide a linear dynamic range of 0.2 – 20 mg/L TOC with a detection limit of max. 0.2 mg/L. The TOC limit value in plastic materials of construction used in packaging systems is NMT (not more than) 5 mg/L (USP 661.1) and in plastic packaging systems for pharmaceutical use it is NMT 8 mg/L (USP 661.2).
For the analysis, purified water extractions from polymer packaging materials have to be prepared under described conditions and to be tested for TOC within four hours after preparation according to USP <643>. USP <643> represents the general method for TOC testing in pharmaceutical applications and provides guidance on how to qualify the analytical technique for use as well as guidance on how to interpret instrument results for use as a limit test (e.g., the 500 ppb limit for WFI – water for injection).
This application note describes the procedure for TOC analysis according to USP <661.1> and <661.2> on the TOC analyzers of the multi N/C pharma series the multi N/C pharma UV, the multi N/C pharma HT and the multi N/C 3100 pharma and proves their outstanding performance for these test methods.

Materials and Methods

Samples and Reagents

A sample of a plastic packaging system for pharmaceutical use and several samples from plastic materials of construction used in such packaging systems were prepared and analyzed according to USP guidelines.

Plastic materials of construction used in packaging system:

- Polyethylene, cyclic olefins and polypropylene
- Polyethylene terephthalate and polyethylene terephthalate G
- Plasticized Polyvinyl chloride

Sample Preparation

The samples were extracted as per USP guidelines. The details of sample preparation are given for each type of material.

Polyethylene, cyclic olefins and polypropylene

25 g of the test material are placed in a borosilicate glass flask and boiled under reflux conditions with 500 mL of purified water for 5 hours. The cooled extracting solution is to be filtered through a sintered-glass filter. The filtrate is collected in a 500 mL volumetric flask and made up to volume with purified water. This solution has to be used within 4 hours of preparation for TOC measurement.

Polyethylene terephthalate and polyethylene terephthalate G

10 g of the test material are placed in a borosilicate glass flask and heat at 50 °C with 200 mL of purified water for 5 hours. After cooling, the solution is decanted into a 200 mL volumetric flask and made up to volume with purified water. This solution has to be used within 4 hours of preparation for TOC measurement.

Plasticized Polyvinyl chloride

25 g of the test material are placed in a borosilicate glass flask, 500 mL of purified water added and the flask's neck covered with aluminum foil or a borosilicate beaker. The flask is heated in an autoclave at 121 ± 2 °C for 20 minutes. After cooling, the solution is decanted into a 500 mL volumetric flask and made up to volume with purified water. This solution has to be used within 4 hours of preparation for TOC measurement.

Plastic packaging systems for pharmaceutical use

The packaging system is filled to its normal capacity with purified water and closed by using the normal means of closure or otherwise with an inert closure. The packaging system is heated in an autoclave at 121 ± 2 °C for 30 minutes. If heating at 121 °C leads to the deterioration of the container, heat treatment at 100 ± 2 °C for 2 hours or at 70 ± 2 °C for 24 ± 2 hours can be applied. After cooling the filled packaging system, its content is emptied and used within 4 hours of preparation for TOC measurement. For this testing method a blank standard has to be prepared by heating purified water in a borosilicate glass flask closed with an inert closure, under the same temperature and time conditions as for sample preparation.

Calibration

The analyzers of the multi N/C pharma series were calibrated for NPOC in the range from 0.1 to 20 mg/L with standard solutions prepared from a 1000 mg/L sucrose stock solution. A multi-point calibration type was used. The calibration curve and its characteristics are presented in Figure 7. An outstanding linearity could be demonstrated throughout the whole calibration range from 0.1 to 20 mg/L for all three analyzer models of the multi N/C pharma series.

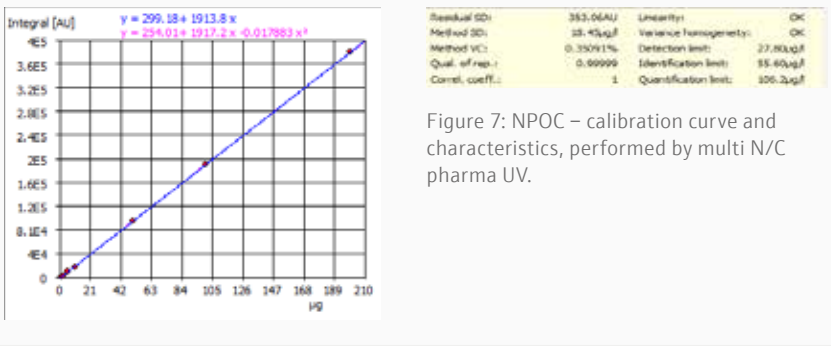


Figure 7: NPOC – calibration curve and characteristics, performed by multi N/C pharma UV.

Instrumentation

The analysis was performed on the multi N/C pharma UV, the multi N/C pharma HT and the multi N/C 3100 pharma.
The method settings shown in Table 7 were used to determine the TOC content.

Table 7: Method settings

	multi N/C pharma UV	multi N/C pharma HT, multi N/C 3100 pharma[1)]
Parameter	NPOC	NPOC
Digestion	UV radiation assisted by $Na_2S_2O_8$	High-temperature oxidation using Pt catalyst at 800 °C
Number of repetitions	Min. 3, max. 4	min. 3, max. 4
NPOC purge time	300 s	300 s
Rinse with sample before injection	3 times	3 times
Injection volume	5 ml	2 ml, 1 ml[1)]

Results

Three customer provided readymade extracts from plastic materials used in packaging systems were measured alongside with a QC check standard after system calibration as described above. Results are displayed in the table and the figure below.

Table 8: Results

Sample ID	NPOC Average [mg/L]	RSD [%]
Sample 1	2.48	0.9
Sample 2	0.984	1.3
Sample 3	8.72	0.5
QC check (2.0 mg/L)	2.04	0.7

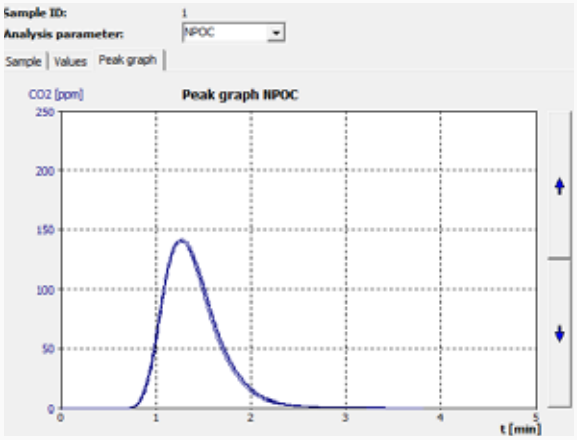


Figure 8: TOC peak graph for sample 1.

Conclusion

This application note clearly demonstrates that the applied TOC analyzers of the multi N/C pharma series provide the required performance characteristics to comply with the USP standards for TOC testing in plastic packaging systems for pharmaceutical use and their materials of construction. With their high oxidation power, the FR-NDIR detector and a sophisticated design the instruments even exceed the required specifications providing a linear dynamic range of 0.1 – 20 mg/L.

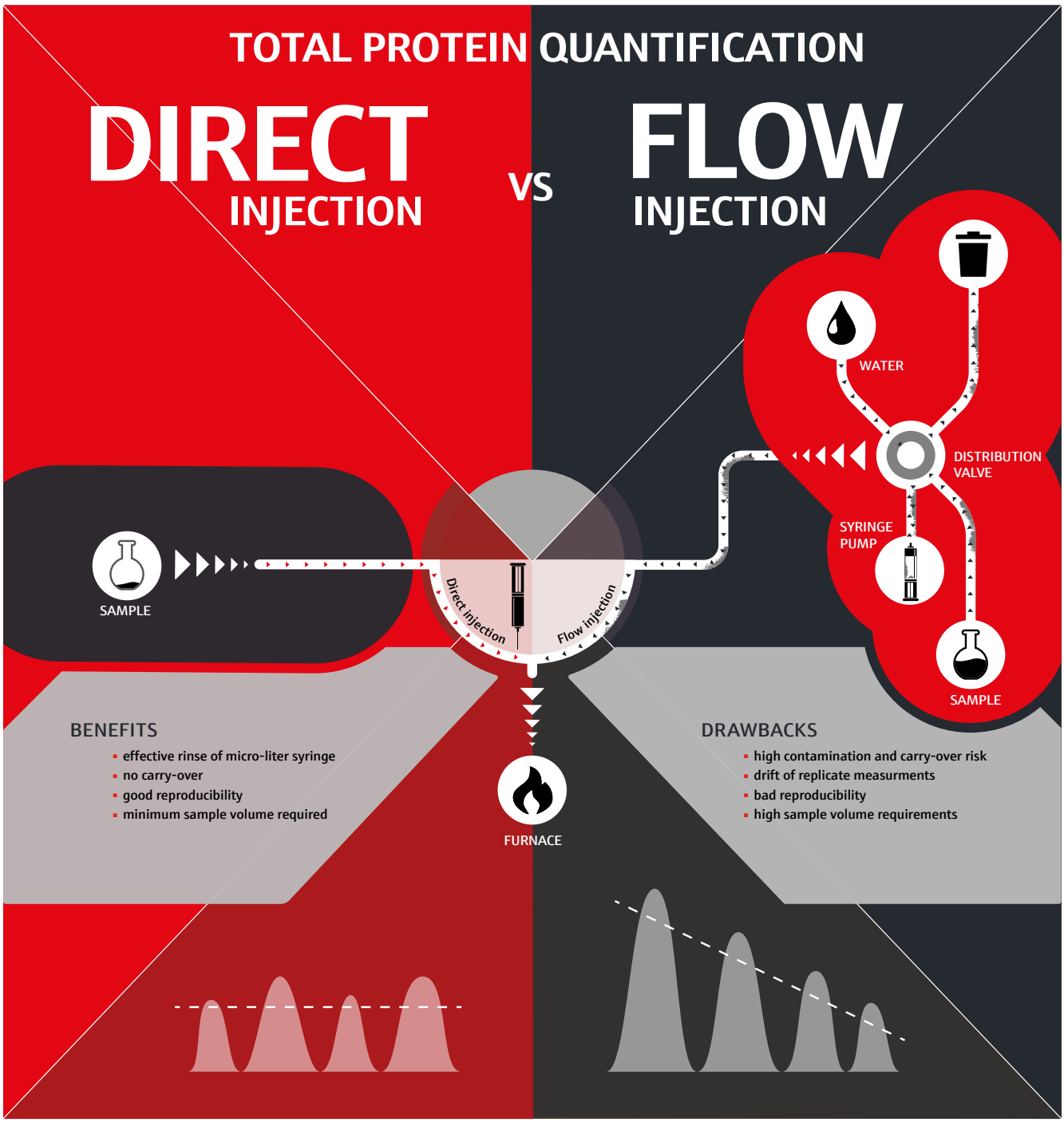
With TOC analyzers from the multi N/C pharma series you are making your lab fit for the new challenges on pharmaceutical TOC testing.

Why You Should Use Direct Injection?

In total protein quality control testing the method of sample handling influences precision and result quality as shown in the figure below.

Flow injection typically suffers from deposition processes of sticky protein sample components to the huge surface areas of long tubing, valve and syringe pump components of the sample introduction system. This leads to system contamination and carry-over issues resulting in drift effects for replicate readings and low precision.

Direct injection by a microliter syringe resolves this issue, since inner surfaces of the syringe body are small and can efficiently be rinsed by plunger-movement and rinse-water. Moreover, the injection needle remains in the hot injection port of the furnace and all sample components evaporate completely, providing a clean needle for the next sample to be processed. This leads to unsurpassed result quality and precision.



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 2100S pharma

Total Protein Determination in Pharmaceutical Products (Vaccines) by TN_b Analysis on multi N/C 2100S pharma

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 which is described in Pharm. Eur. Monograph 2.5.33, Method 7 B: high temperature pyrolysis of the nitrogen compounds in an oxygen atmosphere to nitric oxide (NO) followed by chemiluminescence detection (CLD).

This method description almost coincides with the EN 12260, which describes TN_b determination in environmental water samples by catalytic high temperature combustion and CLD detection of the formed NO molecules. As this is what multi N/C analyzers are designed for they can be applied for TN_b determination in pharmaceutical products as well. The TN_b can than be converted into total protein to determine the level of antigens.

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 [Total Protein] = c [total nitrogen] x 6.25

Materials and Methods

Instrumentation

The analysis was performed on the multi N/C 2100S pharma .The method settings shown in Table 9 were used to determine the TOC content.

Table 9: Method settings

Parameter	multi N/C 2100S pharma
Measurement parameters	TN_b
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

Samples and Reagents

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

After conditioning at room temperature the liquid samples were directly transferred into 2 mL sample vials using a micro pipet and covered with snap-caps. 75 µL of sample aliquots were transferred into the furnace of the analyzer by aid of the micro liter 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 (CLD).The measurement sequence was supported by the auto sampler AS 60 with automatic magnetic stirring of the sampling position and syringe wash station.

Calibration

The multi N/C system was calibrated from 5 to 60 mg/L for total bound nitrogen (TN_b) with a multi-point calibration using a BSA protein standard solution. This is shown in Figure 9. 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.

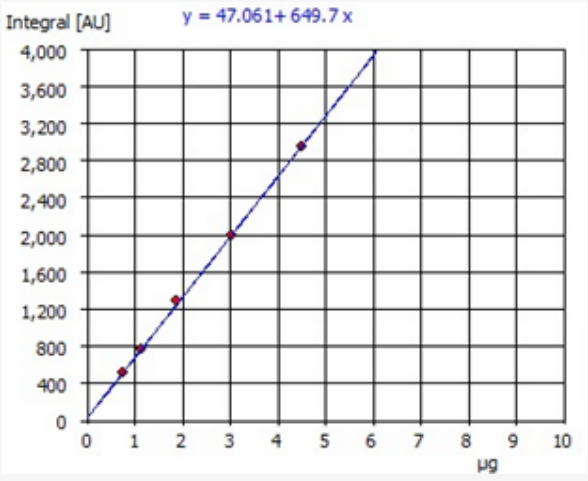


Figure 9: Example of TN – BSA Calibration curve and characteristics range 5 – 60 mg/L N

Results

Each sample has been measured at least three times.

Table 10: Liquid samples

Sample ID	Mean value TN_b [µg/mL] ± RSD [%] (1. vial)	Mean value TN_b [µgm/L] ± RSD [%] (2. vial)	Mean value TN_b [µg/mL] ± RSD [%] (3. vial)
10 ppm Nitrogen Std	10.77 ± 0.14	10.80 ± 0.51	10.74 ± 0.19
20 ppm Nitrogen Std	20.02 ± 0.20	20.05 ± 0.27	19.98 ± 0.24
30 ppm Nitrogen Std	30.31 ± 0.43	30.35 ± 0.28	30.42 ± 0.30
40 ppm Nitrogen Std	40.59 ± 0.27	40.62 ± 0.42	40.65 ± 0.35
50 ppm Nitrogen Std	50.74 ± 0.11	50.96 ± 0.13	50.95 ± 0.29
30 ppm BSA checking Std	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

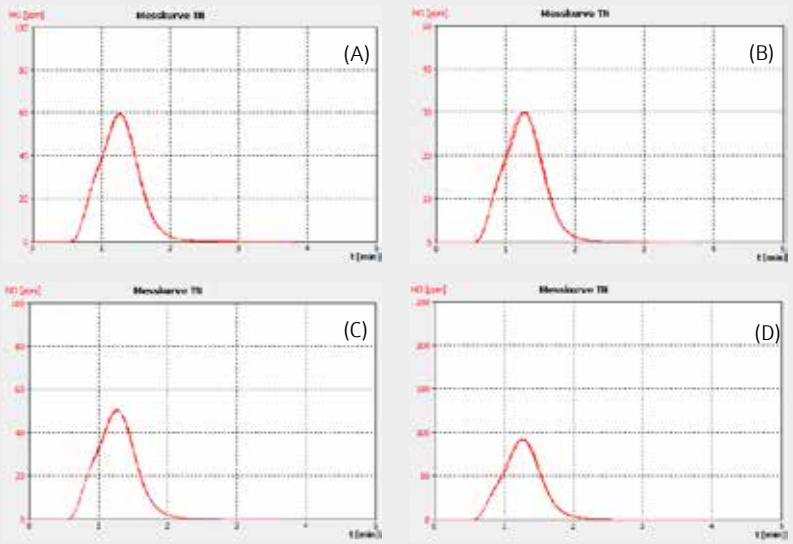


Figure 10: Measuring curves TN of
(A) Example 30 ppm BSA checking Std
(B) Sample A
(C) Sample B
(D) Sample C

Conclusion

All samples were measured with very good repeatability and low RSD's.

The clear advantage of a direct injection system using a micro liter 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 are required.

multi N/C 2100S pharma equipped with a CLD detector for total nitrogen determination is the optimum system to perform total protein analysis for such samples according to the catalytic high temperature digestion method described in Pharm.Eur. 2.5.33 Method 7B.

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.

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