

The Advantages of TOC Analysis Over HPLC for Cleaning Validation

Part 2 of 2

General TOC (Total Organic Carbon) Theory

All TOC analyzers perform two functions: the oxidation of organic carbon in water to carbon dioxide (CO₂) and the measurement of the CO₂ produced. TOC can be used to quantify most of the impurities and residues in equipment that was not cleaned properly, and measures all carbon-containing compounds: APIs (Active Pharmaceutical Ingredients), cleaning agents, proteins, and intermediates. Analytical technologies utilized to measure TOC share the objective of completely oxidizing the organic molecules in an aliquot of sample water to CO₂, measuring the resultant CO₂ levels, and expressing this response as carbon concentration. All technologies must discriminate between the inorganic carbon, which may be present in the water from sources such as dissolved CO₂ and bicarbonate, and the CO₂ generated from the oxidation of organic molecules in the sample. TOC is determined by subtracting the measured inorganic carbon (IC) from the measured total carbon (TC), which is the sum of organic carbon and inorganic carbon: $TOC = TC - IC$.¹

TOC analyzers are differentiated by the methods used to oxidize organics in the water sample and to detect the resulting CO₂ concentration within the sample. Different methods of detection can have a substantial effect on the accuracy of the sample being analyzed, thus affecting the cleaning validation testing process.

TOC Oxidation Technologies

All TOC analyzers on the market use one of two methods for oxidizing organic compounds and converting them to CO₂ gas: combustion or ultraviolet (UV) light with persulfate oxidizer.

The combustion technique uses temperatures of at least 600° C in a stream of nitrogen, oxygen, or air. With the combustion method, a catalyst also is used to aid in the oxidation step. Catalysts used in this methodology are typically cupric oxide, platinum, or cobalt oxide.

The UV Persulfate oxidation methodology utilizes UV light to dissolve organics to fully oxidize to CO₂. The sample is exposed to UV light from a mercury vapor lamp source within the instrument, converting the organics to CO₂ gas. For samples or compounds greater than 1 ppm, persulfate is added and mixed to the sample stream to ensure oxidation on negatively charged hydroxyl (HO-) radicals generated from the irradiation of the sample. Persulfate is a powerful oxidizing agent that, in the presence of UV radiation, can produce sulfate and hydroxyl radicals capable of completely oxidizing organic compounds to CO₂.

TOC Detection Methods

In order to detect the concentration of CO₂, the analyzer needs a detection method to differentiate other molecules from the CO₂ present in the sample. Two current detection methods include Non-Dispersive Infrared (NDIR) or a measurement of conductivity.

NDIR technologies for gas measurement rely on the energy absorption characteristics of each gas in the infrared region of the spectrum. A TOC analyzer that uses the NDIR technique passes infrared through two identical tubes to a detector. The first tube serves as a reference cell and is filled with a non-absorbing gas, like nitrogen. The second cell is used for measurement of the gas sample.

The conductivity detection methodology uses conductivity sensors to determine the concentration of CO₂ via a conductivity calculation. In order to calculate TOC, the aqueous solution passes through two conductivity sensors, measuring the total carbon concentration (TC) in one and the inorganic carbon (IC) concentration in the other. The TOC concentration within the sample is then calculated.

The NDIR method can quantify 0.004–50,000 ppm of carbon in a sample, whereas the conductivity method can quantify as low as the ppb (part per billion) level. In general, NDIR and conductivity detectors are sensitive to low



amounts of TOC, but are subject to ionic interference. This factor is mitigated by use of semi-permeable membranes that are selectively permeable only to CO₂.

Differentiating Factors of Sievers* TOC Technology

The combination of UV persulfate oxidation with a unique, selective CO₂ membrane technology is one of the many factors differentiating the Sievers family of TOC analyzers from more conventional TOC technologies like combustion NDIR techniques. The Sievers technology consistently results in a more accurate TOC reading.

In Sievers' membrane-based conductometric method, the selective CO₂ membrane within the CO₂ transfer module provides a protective barrier to interfering ions, enabling CO₂ to pass freely across the membrane while blocking interfering compounds and oxidized by-products. The selective membrane eliminates the opportunity for background interference and clogging from non-carbon based compounds and by-products.

Cleaning validation applications offer a challenging situation because the TOC concentrations of various samples are at times unknown, making optimal analysis conditions difficult to achieve. The following differentiating factors ensure unsurpassed analytical results with use of the UV persulfate-membrane conductometric technology.

Autoreagent Feature for Complete Oxidation

To ensure complete oxidation of the cleaning validation sample, the Sievers 900 family of analyzers includes an Autoreagent feature for optimized flow rates of acid and the persulfate oxidizer.

Non-Catalytic Combustion Method

The non-catalytic combustion method eliminates human error in determining how much catalyst must be added to the combustion reactor (dependent upon the concentration of carbon in the sample). The combustion oxidation method can produce toxic gas. Some TOC analyzers do not trap potentially harmful gas, which can be created with the presence of chlorides in the cleaning validation sample.

Absence of NDIR Detector

NDIR detectors need time to warm up (30 to 45 minutes), thus contributing even more downtime and sample backlog.

Calibration with the NDIR technology needs to be performed frequently (hourly or daily), depending upon the concentration of carbon in cleaning validation samples. Calibration drift is commonly observed with such detectors. Six to 10% of an NDIR instrument's operation time is dedicated to calibration.

No Use of Carrier Gases

Carrier gases for a NDIR detector can be costly and contribute to high TOC backgrounds by leaks and unstable calibration. Contamination of the carrier gas also could pose a challenge and contribute to high carbon backgrounds.

Superior Sensitivity and Recovery

Conductivity cells within Sievers TOC Analyzers are made of high-purity quartz, providing greater stability and 0.03 ppb levels of detection. **Figure 1** and **Table 1** demonstrate the sensitivity and TOC recovery of Bovine Serum Albumin (BSA) in comparison with the traditional combustion-NDIR TOC technology.

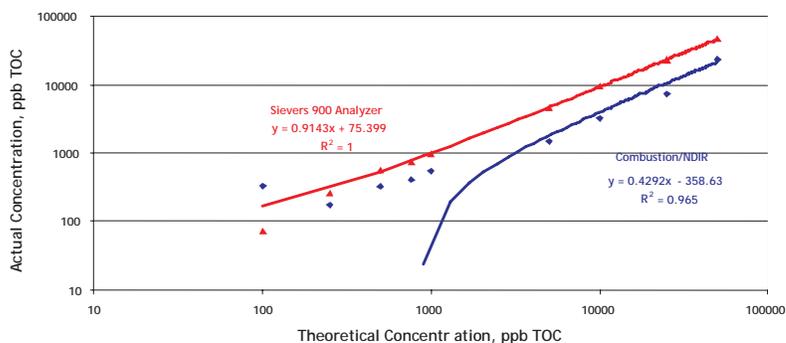


Figure 1. Bovine Serum Albumin (BSA) TOC Percent Recovery Comparability Study

Table 1. Bovine Serum Albumin (BSA) TOC Percent Recovery Comparability Study**

Theoretical Concentration, ppb TOC	Sievers® 900 Results Average TOC Percent Recovery	Combustion/NDIR Results Average TOC Percent Recovery
100 ppb	74.7 ppb = 75%	339.7 ppb = 340%
250 ppb	264.7 ppb = 105%	173.0 ppb = 70%
500 ppb	564.3 ppb = 113%	402.1 ppb = 80%
750 ppb	751.2 ppb = 100%	556.3 ppb = 74%
1000 ppb	968.7 ppb = 97%	556.3 ppb = 56%
5000 ppb	4646.7 ppb = 93%	1508 ppb = 30%
10000 ppb	9390 ppb = 94%	3285 ppb = 33%
25000 ppb	23266.7 ppb = 93%	7183 ppb = 29%
50000 ppb	46000 ppb = 92%	23025 ppb = 46%

**Comparability study conducted on fully calibrated instruments. System suitability tests were performed prior to analysis and passed. The same BSA stock solution was prepared and used on both instruments. The study was conducted within a controlled analytical environment and no deviations occurred to the instruments during the analysis.

Why it is Time to Switch to Sievers TOC for Cleaning Validation

HPLC is a lengthy analysis and delays cleaning validation analysis within the laboratory. This can lead to downtime of hours or days, contributing to high costs and limiting the amount of product reaching patients. Some examples have demonstrated downtime costs in excess of \$1 million per day for some pharmaceutical manufacturers. **Table 2** provides a detailed comparison of the Sievers 900 TOC Analyzer to both combustion/catalyst NDIR and combustion NDIR, including estimated monthly operating cost.

TOC is a fast and simple analytical method for detecting low levels of organic compounds, and allows for detection of contamination not possible via HPLC. TOC has been shown to reduce downtime and method validation time by more than 75% over conventional methods. With the FDA's recent guidance to enhance and modernize the regulation of pharmaceutical manufacturing (**lcGMP's for the 21st Century**), gains in quality and efficiency have led to a growing interest in using TOC analysis for cleaning validation over a specific analytical method like HPLC.²

References

¹ USP <643> Total Organic Carbon.

² Andrew W. Walsh contributed to the content of this application note.

Table 2. TOC Method Comparison

TOC Analyzers Used for Cleaning Validation	Sievers* 900	Combustion/Catalyst NDIR	Combustion NDIR
Oxidation Method	UV persulfate	Combustion (680-1,000° C) catalyst oxidation	680° C combustion oxidation
Detection Method	Membrane conductometric	NDIR	NDIR
Detection Range	0.03 ppb - 50 ppm	TC : 0-25,000 ppm IC : 0-30,000 ppm	4 ppb-25,000 ppm (TOC) 4 ppb-4,000 ppm (TC-IC)
Detection Limit	0.03 ppb	4ppb	4ppb
Accuracy	±2%	CV ±1.5%	--
Precision	1% RSD	--	--
Measuring Item	TOC, TC, IC	TC, IC, TOC, NPOC	TOC (NPOC), TC-IC, TC, IC
Sample Volume	0.5 µL/min	10-2,000 µL	<2mL
Analysis Time	4 minutes	TC : 3 min. or more IC : 3 min. or more	9-12 minutes
Calibration	Annually	Hourly/daily/weekly	Hourly/daily/weekly
Time to Perform Calibration	Single-point: 1.5 hours Multi-point: 5.5 hours	3 hours	5 hours
Time/Year Calibrating	Single-point: 1.5 hours Multi-point: 5.5 hours	150 hours	250 hours
Carrier Gases	n/a	High-purity air 1,440L/mo. (in case of 8H/D x 5D/W)	High-purity air (>99.98%) or High-purity dry N2
Reagents	Acid oxidizer	Hydrochloric acid IC reagent	Hydrochloric acid
On-Line Measurement	Yes	No	No
Off-Line Measurement	Yes	Yes	Yes
Portability	Yes	No	No
Consumables	Acid Oxidizer UV Lamp DI Resin Bed Pump Tubing	Platinum catalyst Carrier gas Acid IC reagent etc.	Carrier gas Acid etc.
Monthly Operating Cost	\$175	\$530	\$540

* Trademark of General Electric Company; may be registered in one or more countries.

For more information, visit www.geinstruments.com. Find a sales partner near you through the "Contact Us" Section.



USA

GE Analytical Instruments
6060 Spine Road
Boulder, CO 80301-3687 USA
T +1 800 255 6964
T +1 303 444 2009
F +1 303 444 9543
geai@ge.com
www.geinstruments.com

Europe

Unit 3 Mercury Way
Urmston, Manchester, M41 7LY
United Kingdom
T +44 (0) 161 866 9337
F +44 (0) 161 866 9630
generaluk.instruments@ge.com