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Paper

The Right Analytical Method for the Right Application: TOC Analysis for Cleaning Validation

Advances in technologies and mounting manufacturing costs are leading the global pharmaceutical industry to evaluate alternate means to enable greater efficiency and productivity. In this highly competitive industry, it is vital to ensure the highest possible drug quality while eliminating high production costs and unnecessarily lengthy validation work. Interest in Total Organic Carbon (TOC) analysis as a non-specific method for cleaning validation has grown considerably in recent years because specific analytical testing like High Performance Liquid Chromatography (HPLC) has proven to be a bottleneck in the cleaning validation process, significantly contributing to equipment downtime after cleaning.

This paper investigates the advantages of using a non-specific method for cleaning validation over more traditional analytical methods. It also looks at TOC methods and identifies why Sievers TOC technology is well suited to cleaning validation applications. The goal is to help pharmaceutical manufacturers understand how using TOC can result in greater resource productivity, improved yields, reduced equipment downtime, and better bottom lines.*

The Advantages of Using a Non-specific Method (TOC) for Cleaning Validation

An increasing number of companies are utilizing TOC analysis for cleaning validation because it is faster, easier, and more economical than other analytical methods. The TOC method yields high sample throughput and reduces cleaning validation protocol execution time. This is true even with compounds generally thought to be insoluble in water or with bulky proteins common in the biotechnology industry. Furthermore, the FDA accepts the TOC method[1] in its regulatory guidelines for measuring contaminant residues.

During a cleaning validation study it is often necessary to establish acceptance criteria limits based upon more than one target residue or compound. HPLC is limited in that it can determine only one residue for one individual given assay. In cleaning validation, multiple compounds would thus require multiple analytical tests. With these multiple tests, unanticipated contaminants or cleaning agents could potentially be overlooked and unknown peaks could show up on the chromatograph. TOC will detect more than one target compound given it is a non-specific method.

Key Weaknesses of HPLC: Ghost Peaks, Regulatory Scrutiny, and Costly Maintenance

Due to long setup and analysis times, HPLC often contributes one to two days of production downtime before processing equipment can be certified for cleanliness. Unknown peaks and costly maintenance have been the root cause of this downtime. Additionally, HPLC is the most commonly cited analytical method in warning letters issued by the FDA after pharmaceutical facility inspections. Recent warning letter statements referencing HPLC address insufficient detection, failure to identify unknown peaks, failure to calibrate instruments prior to use, lack of linearity testing, instrument accuracy, and failure to perform system suitability prior to analysis.[2]

Insufficient training or qualifications for lab personnel running HPLC instruments are also scrutinized heavily. A recent Warning Letter stated "*…procedures for conducting HPLC testing are inadequate because sample run times and retention times…have not been established in your approved test methods. Our investigator found that your laboratory employees routinely stop chromatographic runs immediately after the active peak has eluted, and as a result, any peak that elutes after the active peak will not be detected.*[3]"

This heightened regulatory scrutiny shows that the FDA is aware of HPLC's disadvantages. This awareness is further indicated in the FDA Guide to Inspections of Pharmaceutical Quality Control Laboratories: *"Sometimes the company's employees have insufficient training or time to recognize situations that require further investigation and explanation. Instead they accept unexplained peaks in chromatograms with no effort to identify them.*[4]*"*

It is no secret that performing HPLC analysis for cleaning validation involves some level of uncertainty. Unknown peaks, also known as "ghost peaks," contribute to this uncertainty, resulting in lengthy troubleshooting time and failed validation operations. Peaks from previous injections, contamination, bubbles, column fouling, worn-out guard columns, and trace contaminants or cleaning agents in the sample are some of the contributing factors that lead to costly replacement of parts on the HPLC unit. For example, worn polymeric fittings or tubing and a contaminated guard column affect peak shape and require replacement. Depending upon the peak shape distortion, guard columns may require weekly or even daily replacement, greatly increasing unplanned maintenance costs.

Cost of Ownership

According to various industry experts, the average capital cost of a TOC analyzer is 30% to 40% lower than that of an HPLC instrument. Most pharmaceutical facilities have a TOC analyzer in place for USP water release purposes. The same analyzer can serve both USP and cleaning validation functions, potentially eliminating the need for a capital purchase entirely. Additionally, industry sources estimate operating costs for TOC analysis to be 40% to 80% less than the cost of a HPLC instrument. This does not account for the additional time required for frequent maintenance, removing contaminates by reconditioning the column, using well-degassed solvents, daily column equilibration, and daily calibration of the detector. Operational costs for HPLC will increase due to unreliable components and the multiple external parts needed to run the instrument effectively.

Associated Production Downtime Costs with HPLC

The "downtime calculator" shown in **Table 1** compares production downtime costs associated with the analytical methods most commonly used in the pharmaceutical industry for cleaning validation. The downtime calculator demonstrates the use of HPLC and TOC in an actual pharmaceutical company that is producing a "blockbuster" therapeutic with 24/7 315 days of production.[5] Annual gross revenues for the product are referenced to be $2.5 billion, with 750 resources contributing to the production of the drug product. By using TOC for cleaning validation, the pharmaceutical manufacturer can reduce production downtime costs by almost 100%.

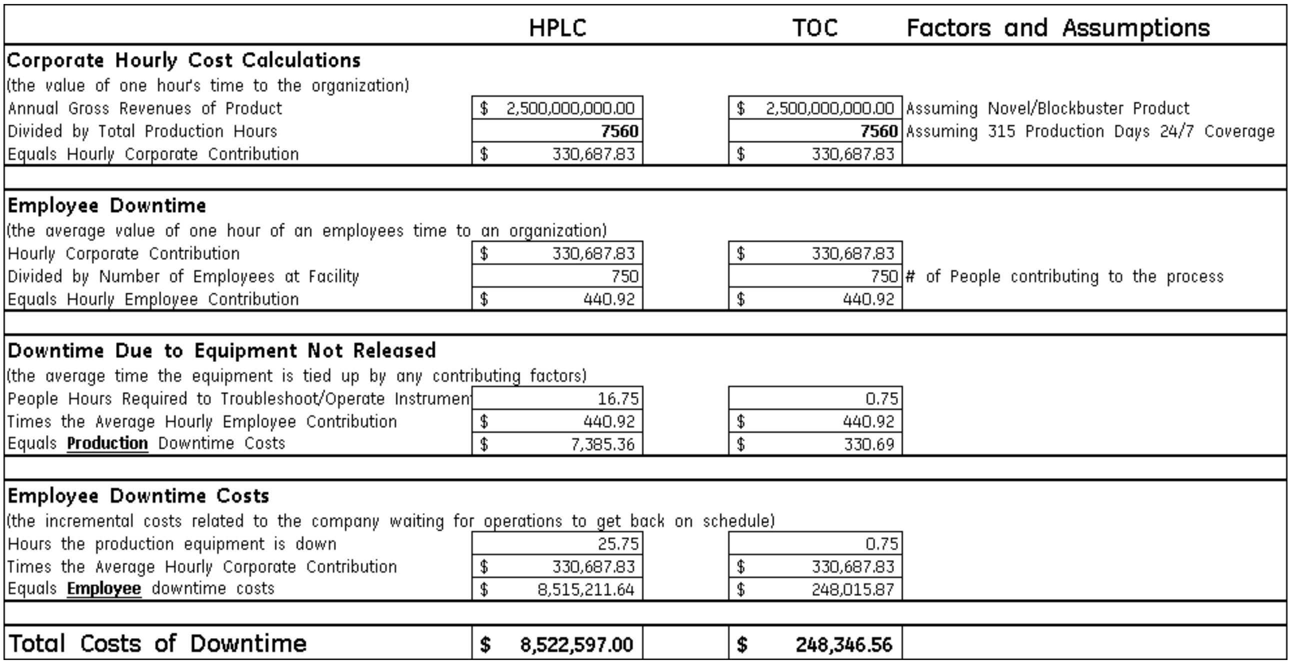
Ease of Use for a Non-Specific Method

An HPLC operation requires attended sample analysis and specially trained personnel. TOC analysis requires no special training and reduces the analytical method development time by 60% to 70%. The use of TOC also reduces the end user decision points, eliminating downtime and human error and streamlining the cleaning validation or verification process. Documentation for TOC analysis is simplified to ensure compliance and promote real-time documentation, thus facilitating rapid approval of lab results for the staged equipment. This allows for quick equipment turnaround time, which is crucial for pharmaceutical manufacturing.

Percent Recovery of Insoluble Organics

With the use of a non-specific method it has been argued that using TOC for cleaning validation did not provide good percent recoveries during cleaning validation stud-

Table 1. Downtime Calculator



	HPLC	TOC	Factors and Assumptions
Corporate Hourly Cost Calculations			
(the value of one hour's time to the organization)			
Annual Gross Revenues of Product	$ 2,500,000,000.00	$ 2,500,000,000.00	Assuming Novel/Blockbuster Product
Divided by Total Production Hours	**7560**	**7560**	Assuming 315 Production Days 24/7 Coverage
Equals Hourly Corporate Contribution	$ 330,687.83	$ 330,687.83	
Employee Downtime			
(the average value of one hour of an employees time to an organization)			
Hourly Corporate Contribution	$ 330,687.83	$ 330,687.83	
Divided by Number of Employees at Facility	750	750	# of People contributing to the process
Equals Hourly Employee Contribution	$ 440.92	$ 440.92	
Downtime Due to Equipment Not Released			
(the average time the equipment is tied up by any contributing factors)			
People Hours Required to Troubleshoot/Operate Instrumen	16.75	0.75	
Times the Average Hourly Employee Contribution	$ 440.92	$ 440.92	
Equals **Production** Downtime Costs	$ 7,385.36	$ 330.69	
Employee Downtime Costs			
(the incremental costs related to the company waiting for operations to get back on schedule)			
Hours the production equipment is down	25.75	0.75	
Times the Average Hourly Corporate Contribution	$ 330,687.83	$ 330,687.83	
Equals **Employee** downtime costs	$ 8,515,211.64	$ 248,015.87	
Total Costs of Downtime	**$ 8,522,597.00**	**$ 248,346.56**	

Source: Pharmaceutical manufacturer

Table 2. Swab Recoveries[6]

Active	Solubility Per Merck Index	Actual Solubility	Solubility as TOC	Recovery HPLC	Recovery TOC
Sulfacetamide	Sparingly Soluble	>10,000 ppm	>5,000 ppm	91.0%	83.1%
Sulfaberizamide	Substantially Insoluble	300 ppm	127 ppm	71.2%	78.0%
Sulfathiazale	Substantially Insoluble	600 ppm	254 ppm	82.4%	86.5%

ies of insoluble organics. Some argue that achieving greater that 50% recovery with a non-specific method analyzing an insoluble compound is very unlikely. **Table 2** demonstrates the swab percent recoveries of three "insoluble compounds" comparing HPLC to TOC. The TOC recovery is from coupons spiked at 4 μg/cm² or approximately 2 parts per million (ppm) in 20 mL of water and demonstrates that the response efficiency is well above 50%.

Using Sievers TOC Analyzers for Cleaning Validation

General TOC Theory

All TOC analyzers perform two functions: the oxidation of organic carbon in water to carbon dioxide (CO_2) and the measurement of the CO_2 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 CO_2, measuring the resultant CO_2 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 CO_2 and bicarbonate, and the CO_2 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.[7]

TOC analyzers are differentiated by the methods used to oxidize organics in the water sample and to detect the resulting CO_2 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 CO_2 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 CO_2. The sample is exposed to UV light from a mercury vapor lamp source within the instrument, converting the organics to CO_2 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 CO_2.

TOC Detection Methods

In order to detect the concentration of CO_2, the analyzer needs a detection method to differentiate other molecules from the CO_2 present in the sample. Two current detection methods include Non-Dispersive Infrared (NDIR) and 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 CO_2 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 to 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 CO_2.

Differentiating Factors of Sievers TOC Technology

The combination of UV persulfate oxidation with a unique, selective CO_2 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 CO_2 membrane within the CO_2 transfer module provides a protective barrier to interfering ions, enabling CO_2 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 Series TOC 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 ten percent of an

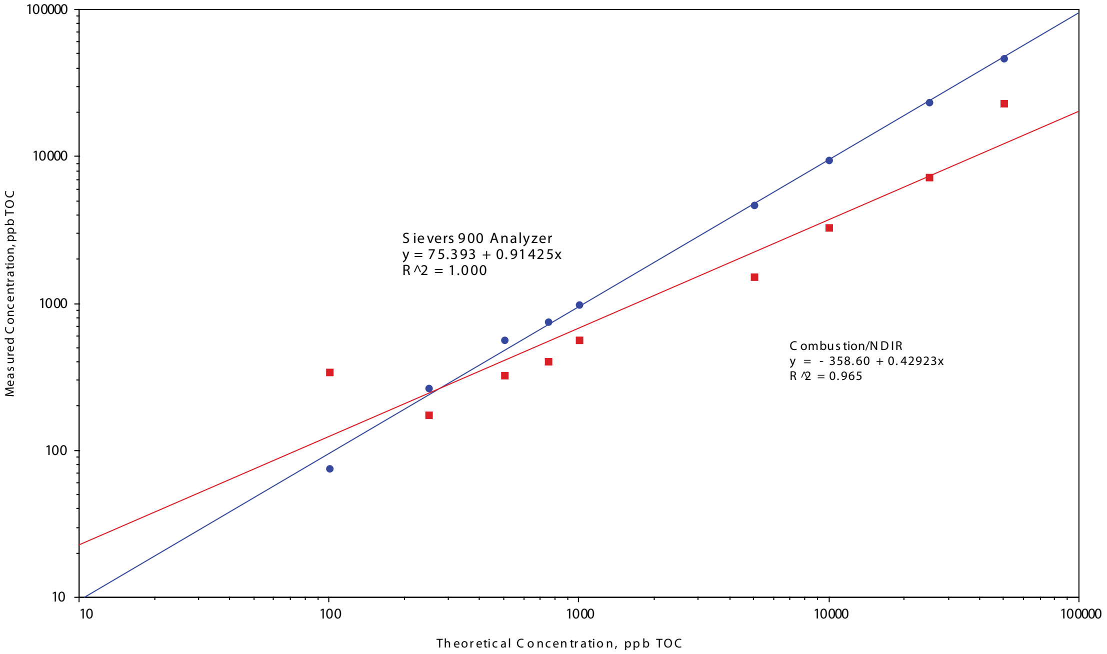


Figure 1. Bovine Serum Albumin (BSA) TOC Percent Recovery Comparability Study

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 3** demonstrate the sensitivity and TOC recovery of Bovine Serum Albumin (BSA) in comparison with the traditional combustion-NDIR TOC technology.

Table 3. Bovine Serum Albumin (BSA) TOC Percent Recovery Comparability Study Data

Theoretical Concentration, ppb TOC	Sievers® 900 Results Average TOC Percent Recovery	Combustion/NDIR Results Average NPOC 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%	323.8 ppb = 64%
750 ppb	751.2 ppb = 100%	402.1 ppb = 54%
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%

Note: 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.

Table 4. 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	4ppb - 25,000ppm (TOC) 4ppb - 4,000ppm (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 ?L/min	10-2,000 ?L	<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

Conclusion

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 4** 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 70% to 80% over conventional methods. With the FDA's recent guidance to enhance and modernize the regulation of pharmaceutical manufacturing (cGMP'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.

References

[1] FDA Website: www.fda.gov/cder/guidance/cGMPs/equipment.htm#TOC

[2] "The Gold Sheet." FDC Reports, March 2005

[3] FDA Website: www.accessdata.fda.gov/scripts/wlcfm/indexdate.cfm

[4] FDA Guidance Document: Guide to Inspections of Pharmaceutical Quality Control Laboratories

[5] This assumes a production shut down for routine maintenance on manufacturing equipment.

[6] Andrew W. Walsh

[7] USP <643> Total Organic Carbon

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