

Application Note

Abstract

In the pharmaceutical industry, various cleaning procedures are performed to make sure there is no cross contamination between product runs of various drugs. The FDA regulates such procedures with Good Manufacturing Practices (GMPs), which requires that the cleaning of drug manufacturing equipment be validated.¹ Total Organic Carbon (TOC) analysis has found wide acceptance in this industry to assist in the validation cleaning procedures, especially clean-in-place (CIP), because TOC concentration levels can be used to track the success of these cleaning procedures.

There has been some speculation that TOC UV/Persulfate analysis is not a suitable technique for this purpose because the presence of hard to oxidize substances would not be detected. This study will compare the TOC analysis using the Teledyne Tekmar Fusion TOC UV/Persulfate system vs. Combustion TOC to verify that both methods are suitable to validate cleaning procedures.

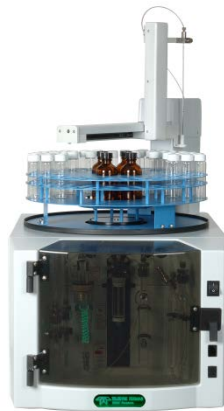


Figure 1: Teledyne Tekmar Fusion UV/Persulfate TOC Analyzer

Introduction

Clean equipment is vital to the pharmaceutical industry due to potential cross contamination between product runs of various drugs. Because of the implications on public health and safety, ensuring cleanliness throughout the production of a drug is critical. To guarantee manufacturing cleanliness, pharmaceutical companies perform various cleaning procedures and the FDA requires that those procedures be validated through Good Manufacturing Practices (GMPs).

Total Organic Carbon (TOC) analysis is a widely accepted way to analyze for residual pharmaceutical components. Teledyne Tekmar TOC analyzers employ two different methods of oxidation: combustion and UV/Persulfate. The Torch TOC analyzer utilizes high-temperature combustion to oxidize organic material in a sample, while the Fusion TOC Analyzer employs UV Persulfate oxidation.

The Torch and the Fusion are both PC-controlled and come equipped with autosamplers allowing unattended analysis of a large number of samples. Both analyzers also use nondispersive infrared (NDIR) sensors for the detection of carbon dioxide.

Experimental-Instrument Conditions

For this study, each TOC analyzer was configured with a default TOC Drinking Water method. **Tables 1 and 2** outline the method parameters. **Table 3** outlines the default TOC Pharmaceutical Water Method which was run on the Fusion to study lower concentrations.

Parameters		Advanced Parameters	
Variable	Value	Variable	Value
Sample Volume	0.3mL	Baseline Stabilization Time	0.25min
Water Chase Volume	1.00mL	Detector Pressure Flow	175mL/min
Dilution	1:1	NDIR Pressurization	50psig
Acid Volume	1.0mL	NDIR Stabilize	0.60min
IC Sparge Time	1.00min	Furnace Temp	750 °C

Table 1: Torch Combustion TOC Instrument Parameters - Default TOC Drinking Water Method

Parameters		Advanced Parameters	
Variable	Value	Variable	Value
Sample Volume	5.0mL	Baseline Stabilization Time	0.70min
Dilution	1:1	Detector Pressure Flow	300mL/min
Acid Volume	1.0mL	NDIR Pressurization	50psig
Reagent Volume	3.0mL	NDIR Stabilize	0.50min
IC Sparge Time	1.00min	Low Level Filter NDIR	Off

Table 2: Fusion UV/Persulfate TOC Instrument Parameters- Default TOC Drinking Water Method

Parameters		Advanced Parameters	
Variable	Value	Variable	Value
Sample Volume	9.0mL	Baseline Stabilization Time	1.60min
Dilution	1:1	Detector Pressure Flow	300mL/min
Acid Volume	0.5mL	NDIR Pressurization	50psig
Reagent Volume	0.8mL	NDIR Stabilize	1.75min
IC Sparge Time	0.5min	Low Level Filter NDIR	Off

Table 3: Fusion UV/Persulfate TOC Instrument Parameters- Default TOC Pharmaceutical Water Method

Sample Preparation

The compounds of interest used in this study include 1,4-benzoquinone, ascorbic acid, citric acid, isonicotinic acid, lignosulfonic acid, l-tryptophan, and sodium hexane-1-sulfonate. Stock solutions of were prepared at 1000ppm Carbon (ppmC) in a 1 L volumetric flask as shown in **Table 4**. From the stock solution, 10.0ppmC solutions were prepared for both the Torch and the Fusion.

The instruments were calibrated with a 20ppmC potassium hydrogen phthalate (KHP) standard. The calibration curve was prepared by using the auto dilution feature to create a 0.0-20ppmC curve on the Torch and a 0.5ppmC to 10.0ppmC curve (n=3) on the Fusion.

Samples were analyzed with both the Torch and the Fusion utilizing the parameters in **Tables 1 and 2**. The standard TOC Drinking Water method parameters were employed for this study.

10.0ppmC solutions were used to verify that the analyzer was able to process the compounds and also to calculate percent yield and percent relative standard deviation (%RSD).

Also on the Fusion, the auto dilution feature was used to prepare a curve fro 0.05-5.0ppmC utilizing the parameters in **Table 3**. Samples at 1.5ppmC were analyzed on the Fusion to verify the analyzer was able to process compounds at low level concentrations and also to calculate percent yield and percent relative standard deviation (%RSD).

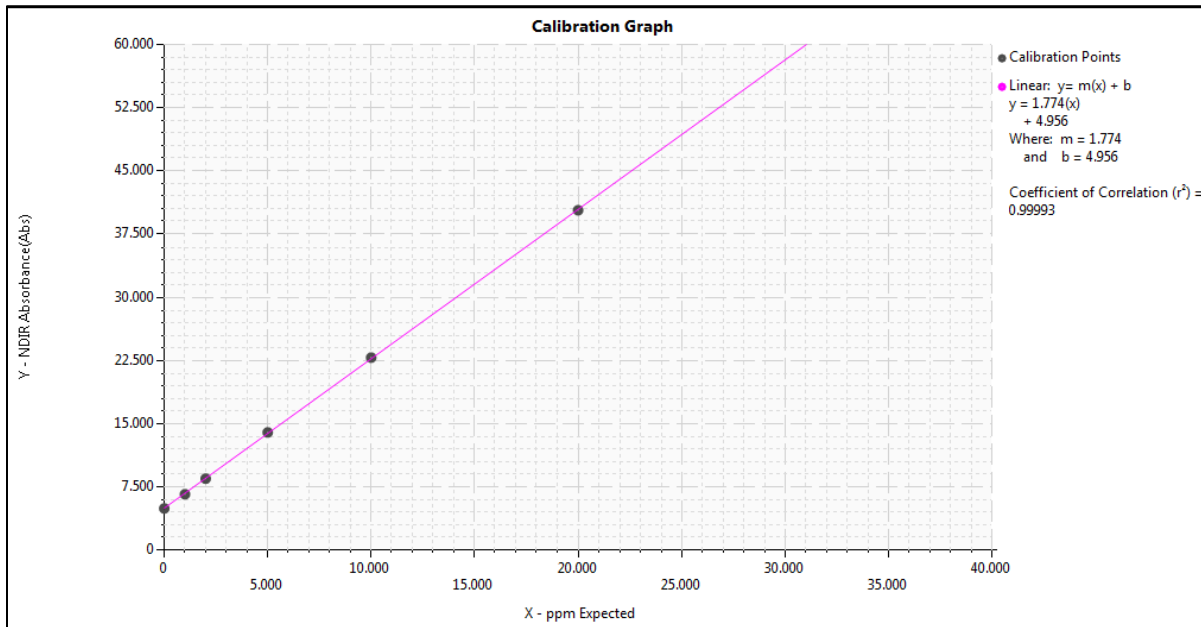
Compound	Molecular Weight	Percent Carbon (%)	g material for 1000ppmC
1,4-Benzoquinone	108.09	66.67	1.490
Ascorbic Acid	176.12	40.92	2.440
l-Tryptophan	204.22	64.70	1.540
Sodium Hexane-1-sulfonate	188.22	38.29	2.612
Isonicotinic Acid	122.12	59.01	1.695
Citric Acid	192.12	37.51	2.666

Table 4: % Carbon Values and Sample Dilutions of the Compounds

Results

Potassium Hydrogen Phthalate (KHP) was used to calibrate the TOC systems. The linear regression factors for the curves (r^2) were evaluated over a range of 0.00-20.0ppmC on the Torch and 0.00-10.0ppmC on the Fusion. **Figures 2, 3, and 4** illustrate the curves developed for this study. **Tables 5-10** show the calibration and check standard data. The relative standard deviation (%RSD) was calculated by analyzing replicate samples for each compound. The percent yield was determined by running samples at known concentrations of 10ppm and comparing them to the curve values. All points on the curve and all samples were run in triplicate.

Torch TOC Calibration Data



**Figure 2: TOC Calibration curve on Torch used to analyze check standards and samples.
 TOC Calibration curve data: $y = 1.774(x) + 4.956$, $R^2=0.99993$**

Pos	Analysis Type	Concentration (ppmC)	Stock Conc. (ppmC)	Dil.	Sample ID	Results (Abs)	Std. Dev. (Abs)	RSD
A	TOC	DI Water [0]	20.0	DI	[TOC] KHP [DI Water]	4.9313	0.1534	3.11%
A	TOC	1.0	20.0	1:20	[TOC] KHP [1.000ppm]	6.6147	0.0849	1.28%
A	TOC	2.0	20.0	1:10	[TOC] KHP [2.000ppm]	8.4630	0.2817	3.33%
A	TOC	5.0	20.0	1:4	[TOC] KHP [5.000ppm]	13.9547	0.1825	1.31%
A	TOC	10.0	20.0	1:2	[TOC] KHP [10.000ppm]	22.8390	0.1285	0.56%
A	TOC	20.0	20.0	1:1	[TOC] KHP [20.000ppm]	40.3380	0.6051	1.50%

Table 5: Torch TOC Calibration Data using Default TOC Drinking Water Method

Torch TOC Check Standards

Pos	BAT	Concentration (ppm C)	Dil.	Sample ID	Min / Max (% dev)	Result	Std. Dev.	RSD
A	TOC	0.1250	1:40	[TOC] KHP [0.1250ppm]	0.1125 / 0.1375 (90% / 110%)	0.1214ppm (PASS)	0.0035ppm	2.87%
A	TOC	5.000	1:4	[TOC] KHP [5.000ppm]	4.5000 / 5.5000 (90% / 110%)	5.4623ppm (PASS)	0.1655ppm	2.97%
A	TOC	10.0000	1:2	[TOC] KHP [10.000ppm]	9.0000 / 11.0000 (90% / 110%)	10.2858ppm (PASS)	0.1550ppm	1.51%

Table 6: Torch TOC Check Standards Data using Default TOC Drinking Water Method

Fusion TOC Calibration Data

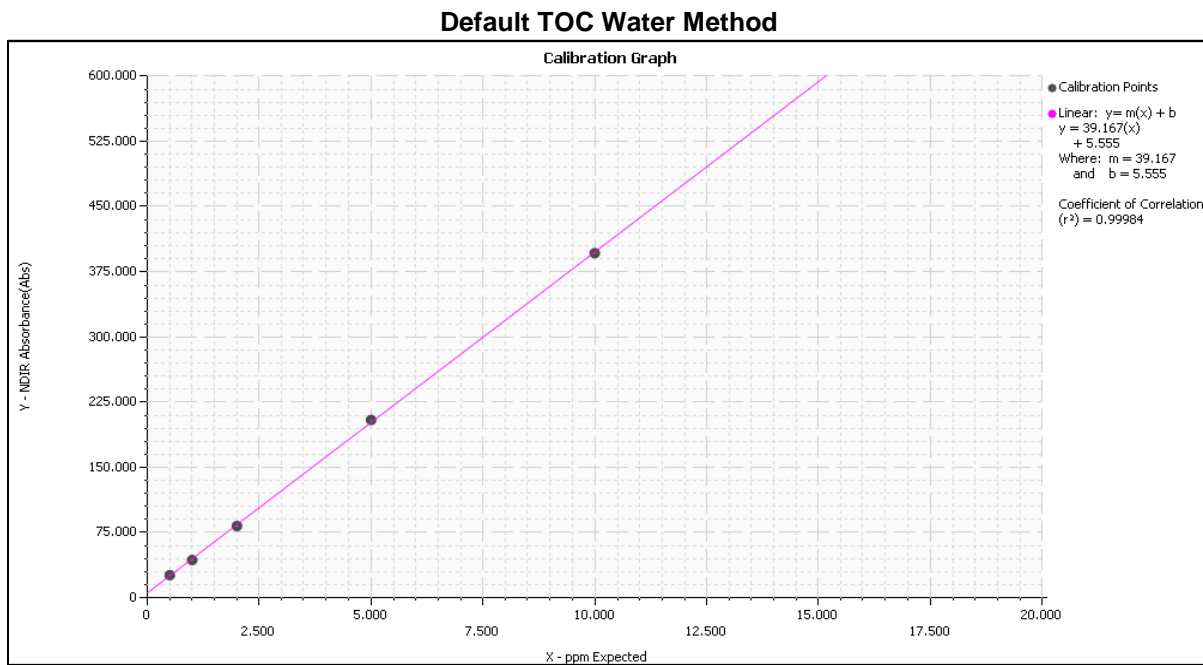


Figure 3: TOC Calibration curve on the Fusion used to analyze check standards and samples. TOC Calibration curve data : $y = 39.167(x) + 5.555$, $R^2=0.99984$

Pos	Analysis Type	Concentration (ppmC)	Stock Conc. (ppmC)	Dil.	Sample ID	Results (Abs)	Std. Dev. (Abs)	RSD
A	TOC	0.5	20.0	1:40	[TOC] KHP [0.500ppm]	25.9900	0.6633	2.55%
A	TOC	1.0	20.0	1:20	[TOC] KHP [1.000ppm]	43.3857	0.0610	0.14%
A	TOC	2.0	20.0	1:10	[TOC] KHP [2.000ppm]	82.4950	0.4343	0.53%
A	TOC	5.0	20.0	1:4	[TOC] KHP [5.000ppm]	204.4133	0.8889	0.43%
A	TOC	10.0	20.0	1:2	[TOC] KHP [10.000ppm]	396.0890	4.0475	1.02%

Table 7: Fusion TOC Calibration Data using Default TOC Drinking Water Method

Fusion TOC Drinking Water Check Standards

Pos	BAT	Concentration (ppm C)	Dil.	Sample ID	Min / Max (% dev)	Result	Std. Dev.	RSD
A	TOC	2.0000	1:10	[TOC] KHP [2.000ppm]	1.8000 / 2.2000 (90% / 110%)	1.9889ppm (PASS)	0.0066ppm	0.33%
A	TOC	4.0000	1:5	[TOC] KHP [4.000ppm]	3.6000 / 4.4000 (90% / 110%)	4.0525ppm (PASS)	0.0173ppm	0.43%

Table 8: Fusion TOC Check Standards Data using Default TOC Drinking Water Method

Default TOC Pharmaceutical Method

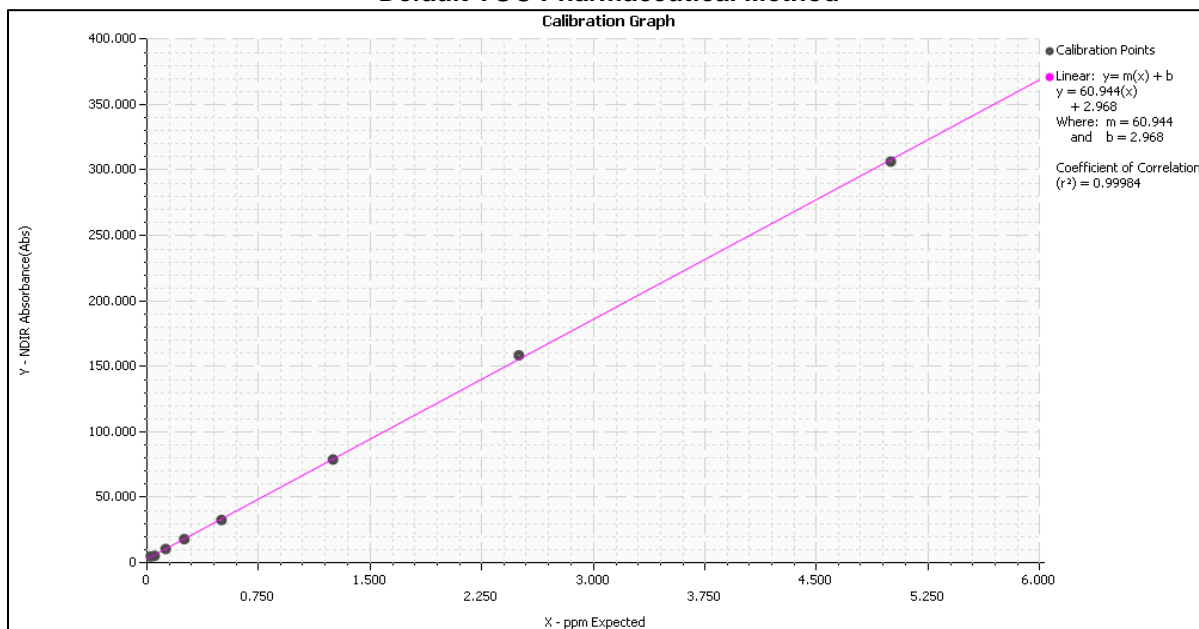


Figure 4: TOC Calibration curve on the Fusion used to analyze check standards and samples. TOC Calibration curve data: $y = 60.944(x) + 2.968$, $R^2=0.99984$

Pos	Analysis Type	Concentration (ppmC)	Stock Conc. (ppmC)	Dil.	Sample ID	Results (Abs)	Std. Dev. (Abs)	RSD
A	TOC	0.050	5.0	1:100	[TOC] KHP [0.050ppm]	5.3277	0.2084	3.91%
A	TOC	0.125	5.0	1:40	[TOC] KHP [0.125 ppm]	10.5113	0.2692	2.56%
A	TOC	0.250	5.0	1:20	[TOC] KHP [0.250 ppm]	18.0483	0.4198	2.33%
A	TOC	0.500	5.0	1:10	[TOC] KHP [0.500 ppm]	32.6780	0.1715	0.52 %
A	TOC	1.250	5.0	1:4	[TOC] KHP [1.250 ppm]	78.8390	0.3931	0.50%
A	TOC	2.500	5.0	1:2	[TOC] KHP [2500 ppm]	158.3907	0.1547	0.10%
A	TOC	5.000	5.0	1:1	[TOC] KHP [5.000 ppm]	306.3223	1.3772	0.45%

Table 9: Fusion TOC Calibration Data using Default TOC Pharmaceutical Method

Fusion TOC Pharmaceutical Water Check Standards

Pos	BAT	Concentration (ppm C)	Dil.	Sample ID	Min / Max (% dev)	Result	Std. Dev.	RSD
A	TOC	0.1250	1:40	[TOC] KHP [0.125ppm]	0.1125/0.1375 (90%/110%)	0.1221ppm (PASS)	0.0015ppm	1.22%
A	TOC	0.5000	1:10	[TOC] KHP [0.500ppm]	0.4500/0.5500 (90%/110%)	0.5017ppm (PASS)	0.0044ppm	0.89%
A	TOC	2.5000	1:2	[TOC] KHP [2.500ppm]	2.2500/2.7500 (90%/110%)	2.48710ppm (PASS)	0.0033ppm	0.13%

Table 10: Fusion TOC Check Standards Data using Default TOC Pharmaceutical Water Method

Sample Analysis

Solutions of the hard to oxidize compounds were made at 10ppmC from a 1000ppmC stock solution. These compounds were then analyzed on both the Torch and the Fusion using the default TOC Drinking Water methods. Solutions of the hard to oxidize compounds were also made at 1.5ppmC from 1000ppmC stock solutions to be analyzed on the Fusion with the Default TOC Pharmaceutical Water method. **Table 11** summarizes the results of the TOC analysis.

Compound	Fusion UV/Persulfate				Torch Combustion	
	TOC Drinking Water		TOC Pharmaceutical Water		TOC Drinking Water	
	% RSD	%Yield	% RSD	%Yield	%RSD	%Yield
1,4-Benzoquinone	0.75	98.90	0.66	94.05	2.18	92.87
Ascorbic Acid	0.83	102.48	0.33	112.79	1.61	105.55
Citric Acid	1.43	112.94	0.26	118.64	1.43	118.31
Isonicotinic Acid	1.40	87.96	0.07	79.74	3.12	105.23
Lignosulfonic Acid	1.10	82.53	0.41	70.47	1.00	75.74
I-Tryptophan	1.37	91.99	0.16	92.52	1.77	110.19
Sodium Hexane-1-sulfonate	1.35	95.33	0.89	99.10	2.00	111.11

Table 11: Summary of Results for 'Hard to Oxidize' Compounds

Conclusions

This study demonstrates the Teledyne Tekmar Fusion TOC analyzer as an effective tool for analyzing the hard to oxidize compounds associated with pharmaceutical clean in place procedures. The Fusion TOC UV/Persulfate system allows for analysis at lower concentration levels in TOC analysis and recoveries still fell within the acceptable range.

Seven compounds were used in this study to illustrate the suitability of UV/Persulfate Oxidation techniques for analysis. Teledyne Tekmar's two current TOC analyzers, the Fusion UV/Persulfate and the Torch Combustion, were compared to verify that both oxidation methods are suitable for the analysis of pharmaceutical compounds classified as hard to oxidize. **Table 4** summarizes the results of this study showing that the Fusion UV/Persulfate TOC Analyzer yields results comparable to a high temperature combustion instrument and is viable for use with hard to oxidize compounds.

References

1. Food and Drug Administration's Current Good Manufacturing Practice Regulations; 21 CFR 211, August 9, 2011.