

TOC Determination of a Clean-in-Place Surfactant Using the Teledyne LABS Fusion UV/Persulfate TOC Analyzer

Steve Proffitt, Applications Chemist, Teledyne LABS

pharmaceutical manufacturing, cleaning validation, total organic carbon, Tekmar Fusion UV/Persulfate TOC analyzer, USP<643>

Introduction

Many guidelines and requirements regulate the cleanliness of the pharmaceutical manufacturing industry. Determining total organic carbon (TOC) levels is an essential tool for ensuring contamination-free equipment for producing safe, high quality pharmaceutical products. Following guidelines and limits according to United States Pharmacopeia (USP<643>) and European Pharmacopeia (Chapter 2.2.44), TOC analyzers can be qualified to be used in cleaning validation applications for determining cleanliness of pharmaceutical equipment. Additionally, The United States Food and Drug Administration (USFDA) has published guidelines for cleaning validation protocols.

Pharmaceutical cleaning processes may involve surfactants as the cleaning agent. Clean-in-place (CIP) procedures must be validated as viable for determining level of cleanliness. TOC analysis of rinse water and swab samples can determine if the equipment has been suitably cleaned for the next process. A commonly used surfactant developed for this purpose will be used for this method validation study.

The surfactant being used is a neutral cleaning booster formulated for the removal of high fat-containing and water free-formulations in pharmaceutical processing equipment. Used as a CIP additive to enhance the cleaning effect of alkaline and acid cleaners, the surfactant is frequently used for the removal of organic soiling such as fats present in residues of creams, ointments, emulsions, oils, and tablet coatings in CIP and manual applications.

To validate TOC analysis as a valid method for determining residues of detergents, this application study shows proof that TOC analysis of a pharmaceutical surfactant using the Fusion UV/Persulfate TOC analyzer is accurate, precise, and linear.

Method

During TOC analysis, the Fusion removes inorganic carbon from the sample in the IC sparger by acid addition and purging the sample with nitrogen. Then the sample is purged in the UV reactor to acquire the TOC result. Sparging a surfactant sample will cause foaming in both the IC sparger and UV reactor. To minimize the foaming and prevent surfactant foam from getting into the exit lines of the sparging vessels, a modified method is used. The default TOC Drinking Water method, which is a pre-set method within the Fusion TOC Teklink software user interface, is modified to use less sample and analyzes samples at a 2:1 dilution ratio (Figure 2).



Figure 1 Fusion UV/Persulfate TOC Analyzer

Parameters		Advanced Parameters	
Variables	TOC	Advanced Variables	Values
Sample Volume (mL)	4	Needle Rinse Volume (mL)	5.0
Dilution	1:2	Vial Prime Volume (mL)	2.0
Acid Volume (mL)	1.0	IC Sample Prime Volume (mL)	2.0
Reagent Volume (mL)	1.0	IC Spurge Rinse Volume (mL)	10
UV Reactor Prerinse	On	Baseline Stabilize Time (min)	0.70
UV Reactor Prerinse Volume	5.0	Detector Pressure Flow (mL/min)	300
Number of UV Reactor Prerinses	1	Syringe Speed Waste	10
IC Spurge Time (mins)	1.00	Syringe Speed Acid	7
Detector Sweep Flow (mL/min)	500	Syringe Speed Reagent	7
PreSpurge Time (mins)	0.20	Syringe Speed DI Water	7
System Flow (mL/min)	350	NDIR Pressurization (psig)	50
		Syringe Speed Sample Dispense	7
		Syringe Speed Sample Aspirate	4
		Syringe Speed UV Dispense	7
		Syringe Speed UV Aspirate	5
		Syringe Speed IC Dispense	7
		Syringe Speed IC Aspirate	5
		NDIR Pressure Stabilize (min)	0.50
		Sample Mixing	Off
		Sample Mixing Cycles	1
		Sample Mixing Volume	10.0
		Low Level Filter NDIR	Off

Figure 2 Modified Method for Analyzing Surfactants

To further prevent excessive foam during analysis, each schedule line is set to analyze one replicate only. If more than one replicate is scheduled per schedule line, simultaneous sparging of all replicates will occur in the IC sparger in which headspace is reduced and excessive foaming is likely to occur.

Calibration

The Fusion was calibrated using the autocalibration feature within the TOC Teklink software. First a 1000 ppm stock standard of potassium hydrogen phthalate (KHP) was prepared by dissolving 2.125 g of KHP in a one liter volumetric flask containing laboratory grade water and diluting to a final volume of 1 liter. Then a 10.0 ppm calibration standard was prepared by pipetting 10.0 mL of the 1000 ppm stock standard into a 1 L volumetric flask and diluting to 1 L with laboratory grade water. A calibration schedule was set up to perform a five-point calibration using calibration points 0.5, 1.0, 2.5, 5.0, and 10.0 ppm. See resulting calibration below in Figure 3.

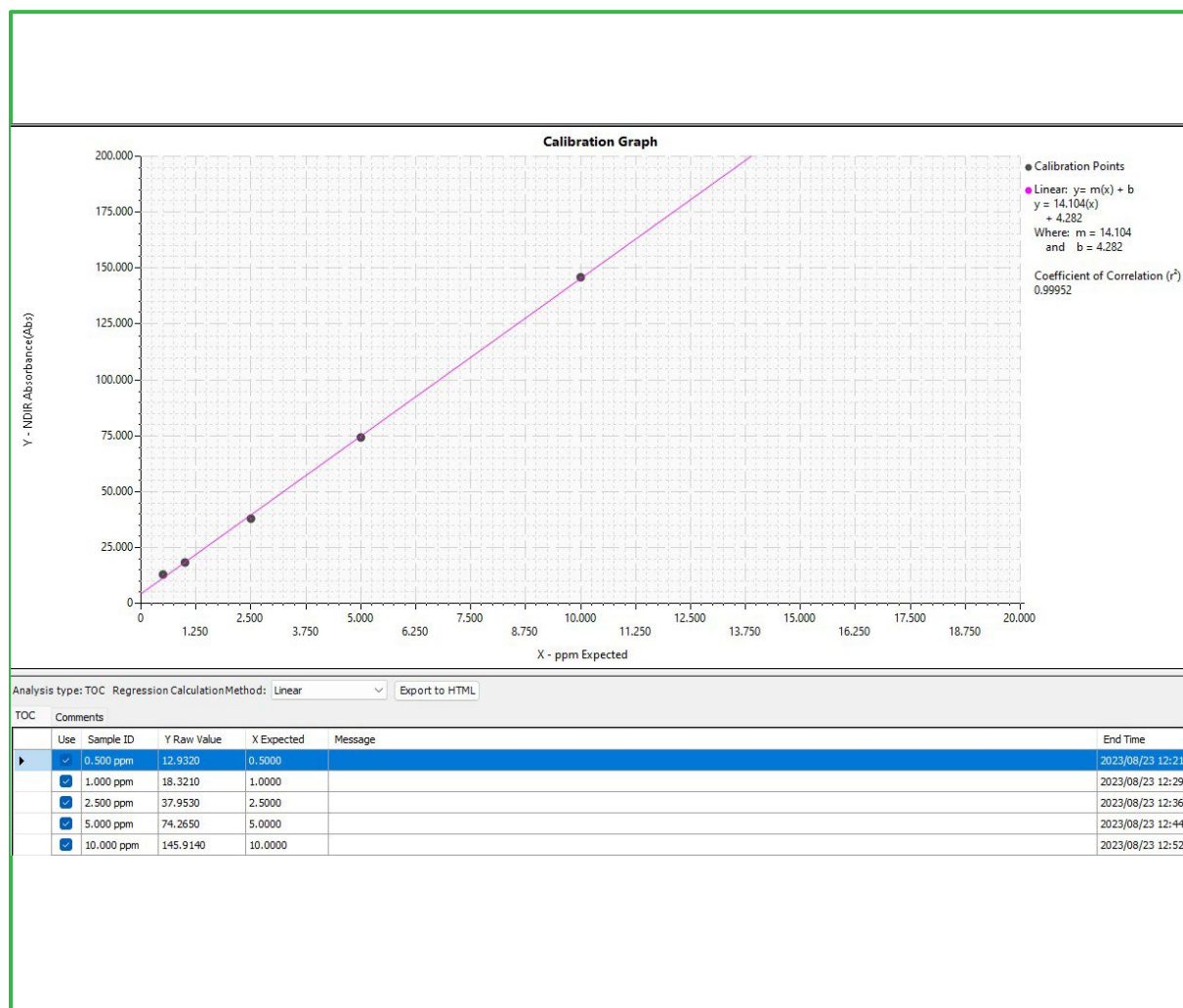


Figure 3 Calibration of Fusion UV/Persulfate TOC Analyzer

Procedures and Results

Validation of Quantitative Determination of Surfactant by TOC Analysis

Validating the quantitative determination of the surfactant by TOC analysis was achieved by analyzing concentrations of the surfactant in the range of 2.5 – 40.0 ppm and verifying linearity has a coefficient of determination (r^2) of equal to or greater than 0.990.

The density of the surfactant is 1.02 g/mL. A 1000 ppm stock solution of the surfactant was prepared by diluting 1.02 g of the surfactant into a 1 L volumetric flask using laboratory grade water. From the 1000 ppm stock solution, individual solutions to be analyzed were prepared directly in 40 mL vials using the calculated values in Table I.

Table I Calculations for Preparing Surfactant Solutions from 2.5 – 40.0 ppm

Concentration of Surfactant (ppm)	mL of 1000 ppm Stock Solution	Final Volume (mL)
2.5	0.1	40.0
5.0	0.2	40.0
10.0	0.4	40.0
20.0	0.8	40.0
30.0	1.2	40.0
40.0	1.6	40.0

A 10 mL pipettor was used to fill each vial with 40 mL of laboratory grade water.

Micro pipettors were used to extract equivalent volumes of water from each vial that will be spiked with 1000 ppm stock solution of the surfactant.

For example, for a 2.5 ppm concentration of surfactant:

1. A 10 mL pipettor was used to fill vial with 40 mL of laboratory grade water.
2. A micro pipettor was used to extract 0.1 mL of water from the vial.
3. A micro pipettor was used to add 0.1 mL of 1000 ppm stock solution to the vial.
4. Vial was capped, inverted, and shaken to ensure adequate mixing was achieved.

This process was repeated for each concentration level applying appropriate volumes.

The TOC content provided by analysis using the Fusion UV/Persulfate TOC analyzer of the dilutions of the surfactant and the results of the linear regression are shown below in Figure 4.

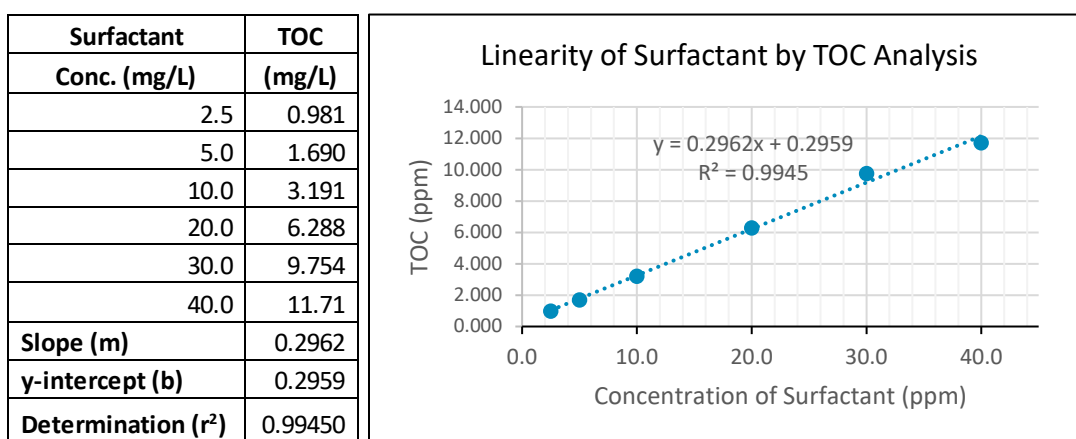


Figure 4 Linear Regression Demonstration of TOC Quantitation of Surfactant

Linear regression of the data provided by the TOC quantitation of the surfactant demonstrates linearity in the range of 2.5 ppm to 40 ppm.

Accuracy

Data provided by manufacturer of surfactant in Table II characterizes the surfactant to be 35.15% carbon.

Table II Characterization Data of Surfactant				
PRODUCT	TOC (g/L)	Density (g/mL)	% TOC (w/w)	Method
Surfactant	358	1.02	35.15	I100.115.01

The mean results of three analyses by the Fusion UV/Persulfate TOC analyzer verifies accurate measurements as compared to the carbon value provided by the surfactant manufacturer. See Table III below.

Table III Calculations for Preparing Surfactant Solutions from 2.5 – 40.0 ppm			
Surfactant Conc. (mg/L)	TOC Mean (mg/L)	True Value (mg/L)	% of True Value
2.5	0.988	0.879	112
5.0	1.664	1.758	94.7
10.0	3.131	3.515	89.1
20.0	6.289	7.030	89.5
30.0	9.740	10.545	92.4
40.0	12.203	14.060	86.8
			Mean % = 94.1

The mean percent recovery value across the linear range of 2.5 ppm to 40 ppm is 94.1%.

Robustness

To demonstrate the robustness of the analytical capability of the Fusion UV/Persulfate TOC analyzer, three sets of quantitation schedules were analyzed over a three week period. The results are captured below in Table IV and Figure 5.

Table IV TOC Quantitation Results Over Three-Week Period						
Date	9/6/2023	9/14/2023	9/18/2023			
Surfactant	TOC	TOC	TOC			
Conc. (mg/L)	(mg/L)	(mg/L)	(mg/L)	Mean	Std Dev	%RSD
2.5	0.981	0.991	0.992	0.988	0.0062	0.63
5.0	1.690	1.681	1.621	1.664	0.0377	2.26
10.0	3.191	3.047	3.155	3.131	0.0749	2.39
20.0	6.288	6.152	6.427	6.289	0.1377	2.19
30.0	9.754	9.428	10.04	9.740	0.3047	3.13
40.0	11.71	12.07	12.83	12.20	0.5731	4.70
Slope (m)	0.2962	0.3005	0.3229			
y-intercept (b)	0.2959	0.1771	0.0595			
Determination (r ²)	0.99450	0.99904	0.99870			

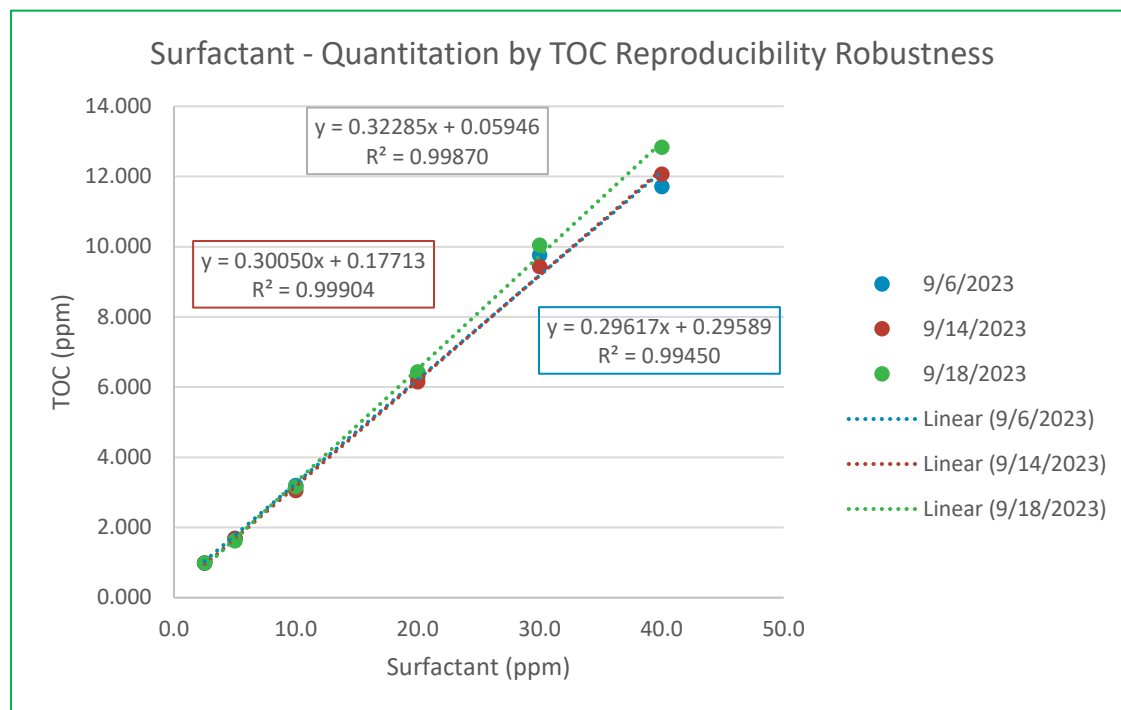


Figure 5 Graphical Comparison of TOC Quantitation Results Over Three-Week Period

In each analytical result, linearity is confirmed with excellent accuracy and precision, validated by low percent relative standard deviation (%RSD) calculations.

Precision

Precision of the analytical method was confirmed by analyzing seven replicates and calculating %RSD. A mid-point concentration of 10.0 ppm of the surfactant was used for this test procedure. Results are in Table V.

Table V TOC Results for Precision Test			
10ppm Surfactant	TOC (mg/L)	True Value (mg/L)	% Recovery
1	3.294	3.515	93.7
2	3.303	3.515	94.0
3	3.303	3.515	94.0
4	3.256	3.515	92.6
5	3.283	3.515	93.4
6	3.235	3.515	92.0
7	3.290	3.515	93.6
mean	3.216	3.515	93.3
std dev	0.025	0.000	0.724
%RSD	0.78	0.00	0.78

The results for the precision test were exceptional with a %RSD of 0.78% calculated for seven replicates. The percent recovery of the TOC value reported from the surfactant manufacturer was also excellent at 93.3%.

Detection Limit

Detection limits come in a variety of forms. For this application note, three different calculations were performed as detailed below:

- Method Detection Limit (MDL)
 - Source: USEPA, 40 CFR Part 136
 - Minimum concentration measured with 99% confidence
 - The MDL for the target analyte must comply with the following rule:
 - The calculated MDL must be less than the spike concentration and greater than $\frac{1}{10}$ the spike concentration ($\frac{1}{10} \text{ spike} < \text{MDL} < \text{spike}$)
 - Calculation: Standard deviation (SD) x Student's t value (SD x 3.14 for 7 reps)

- Limit of Detection (LOD)
 - Source: American Chemical Society (ACS)
 - Lowest concentration statistically different from blank
 - Calculation: 3 x SD

- Limit of Quantitation (LOQ)
 - Source: ACS
 - Level above which quantitative results have specified degree of confidence
 - Calculation: 10 x SD

For all detection limits, spikes were analyzed at 0.5 ppm of the surfactant.

Table VI Detection Limit Results					
0.5ppm Surfactant	TOC (mg/L)	Blank (mg/L)	Adj. Value (mg/L)	True Value (mg/L)	% Recovery
1	0.4386	0.2388	0.1998	0.1757	113.7
2	0.4147	0.2388	0.1759	0.1757	100.1
3	0.4326	0.2388	0.1938	0.1757	110.3
4	0.4020	0.2388	0.1632	0.1757	92.9
5	0.4040	0.2388	0.1652	0.1757	94.0
6	0.4188	0.2388	0.1800	0.1757	102.5
7	0.3936	0.2388	0.1548	0.1757	88.1
mean	0.407		0.176	0.1757	100.232
std dev	0.0161		0.0165	0.000	9.375
%RSD	3.97		9.35	0.00	9.35

Calculated values:

$$\text{MDL} = \text{SD} \times 3.14 = 0.052$$

$$0.05 < 0.052 < 0.5$$

$$\text{LOD} = \text{SD} \times 3 = 0.049$$

$$\text{LOQ} = \text{SD} \times 10 = 0.165$$

The result for calculated MDL at a concentration of 0.5 ppm of the surfactant satisfies the rule required by the USEPA, wherein: $\frac{1}{10} \text{ spike} < \text{MDL} < \text{spike}$.

Validation of the Determination of the Surfactant by TOC Analysis after Rinse Sampling

The procedure for rinse sampling first required spotting modified 25 cm² stainless steel coupons with three contamination levels. Each contamination level was performed in triplicate. The coupon was modified by bending into a shape to funnel the rinsate directly into a 40 mL vial.

A 1000 ppm solution of the surfactant was applied spot-wise to the stainless steel coupons, the coupons were allowed to dry for six hours, then rinsed with four aliquots of 10 mL laboratory grade water into a 40 mL vial for a final volume of 40 mL. See details according to Table VII below.

Table VII Values for Contamination Spotting of Stainless Steel Coupons for Rinse Sampling			
Contamination Level (ppm)	1000.0 ppm Surfactant (mL)	Drying Time (hrs.)	Final Volume (mL)
5.0	0.2	6.0	40.0
10.0	0.4	6.0	40.0
20.0	0.8	6.0	40.0

Results for rinse sampling are shown below in Table VIII and Figure 6.

Table VIII Results for Rinse Sampling								
Date	9/28/2023	9/28/2023	9/28/2023					
Surfactant	TOC	TOC	TOC					
Conc. (mg/L)	(mg/L)	(mg/L)	(mg/L)	Average	Std Dev	%RSD	Cal. Ave.	% of Cal. Ave.
5.0	1.370	1.753	1.594	1.572	0.1926	12.25	1.631	96.38
10.0	3.055	3.154	3.081	3.097	0.0516	1.67	3.069	100.89
20.0	6.195	6.280	6.149	6.208	0.0666	1.07	6.166	100.69
Slope (m)	0.3206	0.3033	0.3041				Ave. % Rec.	99.32
y-intercept (b)	-0.2005	0.1901	0.0598					
Co of Det (r ²)	0.99969	0.99930	0.99994					

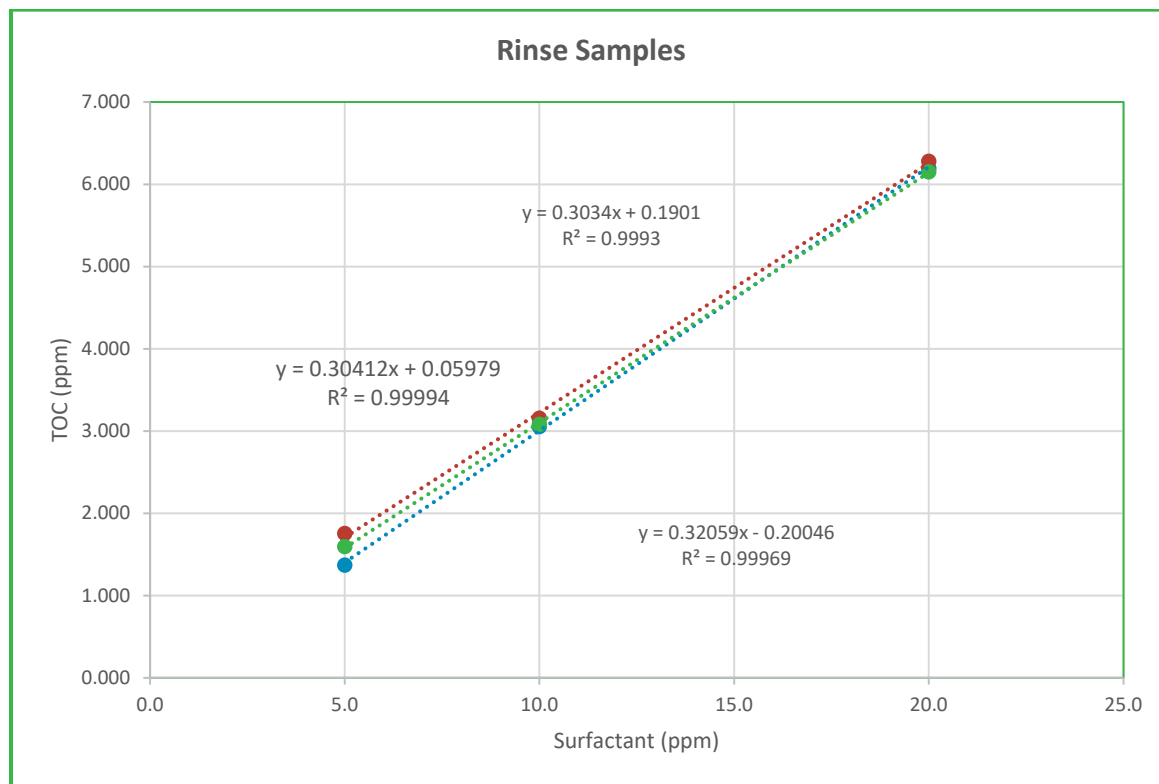


Figure 6 Graphical Representation of Reproducibility and Linearity of Rinse Samples

The results of the rinse sampling show good overall reproducibility with a bit of variance at the low level. Linearity is verified in all three trials with $r^2 > 0.999$. The rinse technique is also validated with the average percent recovery of calibration values at 99.32%.

Validation of the Determination of the surfactant by TOC Analysis after Swab Sampling

The procedure for swab sampling first required spotting 25 cm² stainless steel coupons with three contamination levels. Each contamination level was performed in triplicate. A 2000 ppm solution of surfactant was applied spot-wise to the stainless steel coupons. See details according to Table IX below.

Table IX Values for Contamination Spotting of Stainless Steel Coupons for Swab Sampling			
Contamination Level (ppm)	2000.0 ppm Surfactant (mL)	Drying Time (hrs.)	Final Volume (mL)
5.0	0.1	6.0	40.0
10.0	0.2	6.0	40.0
20.0	0.4	6.0	40.0

The coupons were allowed to dry for six hours. After drying time, a swab was wetted on each side with 10 µL of laboratory grade water. The coupon was swabbed side to side vertically, the swab was then rotated to other side and the coupon was swabbed side to side horizontally as pictured in Figure 7.

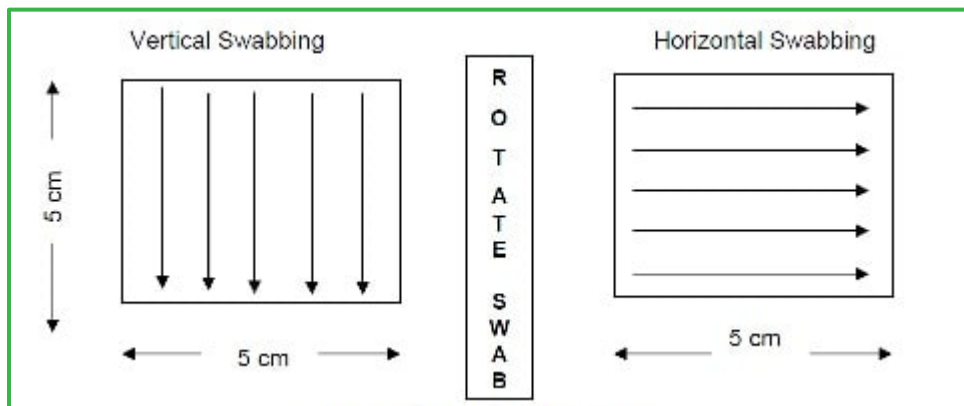


Figure 7 Swabbing Technique

The swab tip was then cut off and transferred into a sampling vial containing 40 mL of laboratory water. Once all sampling is complete, the vials were placed on a shaker table and shaken for 15 minutes. After 15 minutes, samples were allowed to settle while setting up an analysis schedule on the Fusion UV/Persulfate TOC analyzer.

Results for swab sampling are shown below in Table X and Figure 8.

Table X Results for Swab Sampling								
Date	9/28/2023	9/28/2023	9/28/2023					
Surfactant	TOC	TOC	TOC					
Conc. (mg/L)	(mg/L)	(mg/L)	(mg/L)	Average	Std Dev	%RSD	Cal. Ave.	% of Cal. Ave.
5.0	1.003	1.123	1.170	1.098	0.0863	7.86	1.631	67.34
10.0	2.224	2.107	2.281	2.204	0.0884	4.01	3.069	71.80
20.0	4.919	4.856	5.099	4.958	0.1259	2.54	6.166	80.42
Slope (m)	0.2623	0.2526	0.2648				Ave. % Rec.	73.18
y-intercept (b)	-0.3454	-0.2515	-0.2393					
Co of Det (r ²)	0.99943	0.99419	0.99690					

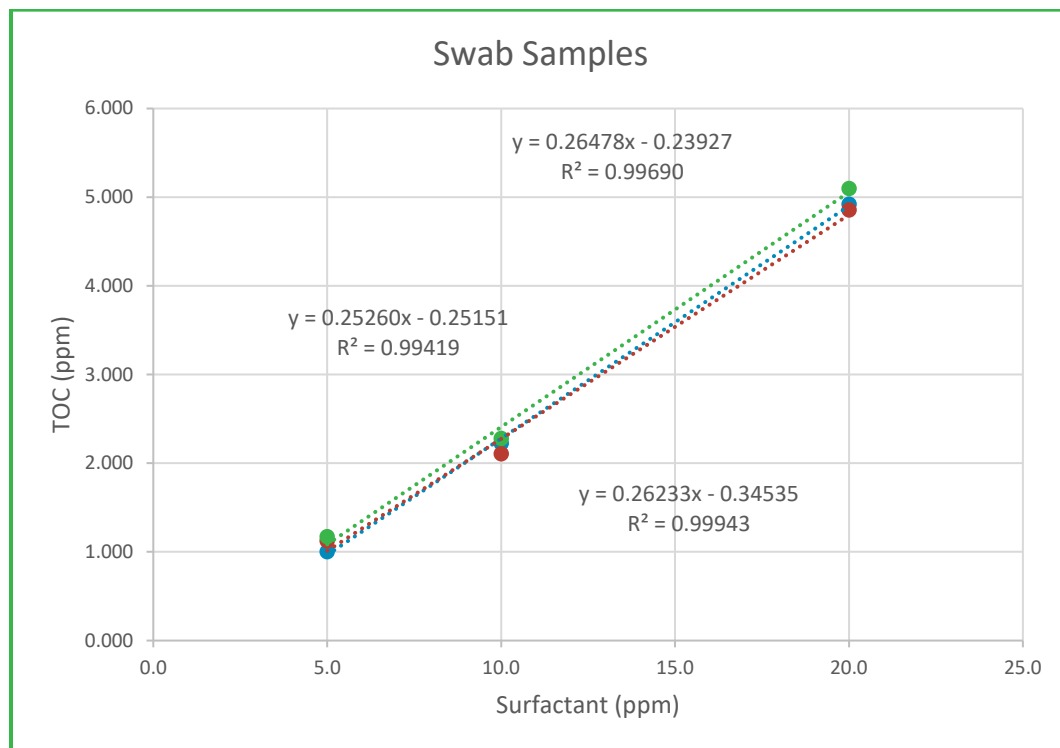


Figure 8 Graphical Representation of Reproducibility and Linearity of Swab Samples

The results of the swab sampling show good reproducibility demonstrated by low percent RSD for replicate sampling. Linearity is verified in all three trials with $r^2 > 0.990$. The swab technique is also validated with the average percent recovery of calibration values at 73.18%. Recoveries are not as high as for rinse sampling due to some adhesion of surfactant to coupon after swabbing completion.

Conclusion

The goal of this application was achieved, which was to validate the suitability of the determination of TOC by a UV/Persulfate analyzer in accordance with USP <643> and Ph. Eur. 2.2.44 as a method for trace analysis of residues of the surfactant. Also proven was the adequacy of the rinse and swab techniques of determining TOC levels on contaminated stainless steel surfaces. The TOC results for all testing were verified to be accurate, precise, and linear.

References

1. United States Pharmacopeia <643> Total Organic Carbon [Revised: 01-May-2021]
2. European Pharmacopeia 6. Edition 2008, 2.2.44, Total Organic Carbon in Water for Pharmaceutical Use
3. Trace Analysis of COSA PUR 88 by Means of TOC Determination in Cleaning Validation Samples after Swab and Rinse Sampling, 100.137.01, TECHPharm GmbH
4. United States Food and Drug Administration, Guide to Inspections – Validation of Cleaning Processes [Revised: 08/26/2014]