

Application Note

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Objective

To determine if the Teledyne Tekmar Fusion UV/Persulfate TOC Analyzer is capable of complying with United States Pharmacopeia (USP) Method <643> Bulk and Sterile Concentrations, using one calibration curve.

Background

USP <643> provides guidelines and requirements for TOC analysis of water for injection and purified water. This method presents a System Suitability Test that compares the recovery of a Standard Solution (r_s) of sucrose (a relatively easy compound to oxidize) to a System Suitability Solution (r_{ss}) of 1,4-benzoquinone (a difficult to oxidize compound). The response of Reagent Water (r_w) is subtracted from each of these solutions' responses to yield a corrected response. The corrected responses are then compared and must be within 15% of each other to verify the system will thoroughly oxidize organic carbon compounds with differing affinities for oxidation. The Response Efficiency must be 85%-115% for the Bulk and Sterile Standards to meet USP <643> requirements using the equation in [Figure 1](#).



Figure 1 Equation from USP <643> to Calculate % Response Efficiency

$$\% \text{ Response Efficiency} = 100[(r_{ss} - r_w)/(r_s - r_w)]$$

Where:

- r_{ss} = Instrument response to the System Suitability Solution (1, 4 Benzoquinone)
- r_w = Instrument response to the Reagent Water Control
- r_s = Instrument response to the Standard Solution (Sucrose)

The Teledyne Tekmar application note, "Simplifying the Process: Automated USP 643 / EP 2.2.44 Purified Water and Water for Injection Testing Using a Next Generation TOC Analyzer" ([viewable here](#)) demonstrated the Fusion's ability to easily pass USP Method <643> requirements with 0.500 ppmC standards and explains the Fusion's helpful software features. There have been recent updates to USP <643> that require not only 0.500 ppmC concentration of sucrose and 1,4-benzoquinone which represents "Bulk Water", but an additional "Sterile Water" concentration level of 8.000 ppmC. The method does not require that both the Bulk and Sterile Standards be calculated against the same calibration, but for ease of use, and to reduce calibration time, one calibration curve for both Bulk and Sterile Standards will be used.

Instrument Method

The typical pharmaceutical or low-level carbon method on the Fusion uses a 9.0 ml Sample Volume, but to incorporate the 8.000 ppmC Sterile Standards of sucrose and 1,4-benzoquinone, the Sample Volume was decreased to 4.5 ml ([Figure 2](#)) in the Fusion software. The UV Reactor Pre Rinse was also set to "On" and the Pre Rinse Volume set to 5.0 ml of rinse water between injections, to reduce carryover.

Figure 2 Method Settings Showing 4.5 ml Sample Volume and 5.0 mL Prerinse Settings

General	Advanced	Comments
Analysis Type (Complex): TOC Calibration: TOC Pharmaceutical Water		
Optimal Sample Range: 0.0024 to 13.4 (ppm)		
Variables		TOC
Sample Volume (mL)	4.5	
Dilution	1:1	
Acid Volume (ml)	0.5	
Reagent Volume (ml)	0.8	
UV Reactor Prerinse	On	
UV Reactor Prerinse Volume	5.0	
Number of UV Reactor Prerinse	1	
IC Sparge Time (mins)	0.50	
Detector Sweep Flow (ml/min)	500	
PreSparge Time (mins)	0.50	
System Flow (ml/min)	500	

System Suitability Schedule

The Calibration Standards ranging from 0.100 ppmC-10.000 ppmC were made from potassium hydrogen phthalate (KHP) according to the procedure in the *Fusion User Manual*. System Suitability Standards of 0.500 ppmC sucrose, 0.500 ppmC 1,4-benzoquinone, 8.000 ppmC sucrose, and 8.000 ppmC 1,4-benzoquinone were also prepared. Both sets of standards were run on the Fusion as shown in Figure 3.

Figure 3 Fusion Sample Schedule Run

Position	Sample Type	Sample ID	Method ID (Calibration ID)	Reps
1	Clean	Clean		1
2	Blank	Reagent/Acid Blank		1
3	Cal Standard	[TOC] 643 user [0.100 ppm]	643 pharma (TOC Pharmaceutical Water)	3
4	Cal Standard	[TOC] 643 user [0.500 ppm]	643 pharma (TOC Pharmaceutical Water)	3
5	Cal Standard	[TOC] 643 user [1.000 ppm]	643 pharma (TOC Pharmaceutical Water)	3
6	Cal Standard	[TOC] 643 user [5.000 ppm]	643 pharma (TOC Pharmaceutical Water)	3
7	Cal Standard	[TOC] 643 user [10.000 ppm]	643 pharma (TOC Pharmaceutical Water)	3
8	Sample		643 pharma (TOC Pharmaceutical Water)	3
9	System Sui...	[ReagentWater] 643 Sterile [Reagent Water]	643 pharma (TOC Pharmaceutical Water)	3
10	System Sui...	[StandardSolution] 643 Sterile [Sucrose (500 ppb)]	643 pharma (TOC Pharmaceutical Water)	3
11	System Sui...	[SuitabilitySolution] 643 Sterile [1,4-Benzoquinone (500 ppb)]	643 pharma (TOC Pharmaceutical Water)	3
12	Sample		643 pharma (TOC Pharmaceutical Water)	3
13	Check Sta...	[TOC] Bulk [8 ppmC Sucrose]	643 pharma (TOC Pharmaceutical Water)	3
14	Check Sta...	[TOC] Bulk [8ppmC 1,4B Benzoquinone]	643 pharma (TOC Pharmaceutical Water)	3
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Calibration Standard Results

The results from the calibration curve yielded a linear regression value of 0.99956. While a linear regression is not required by USP <643>, it is important to have a good linear value (>0.995) to acquire accurate System Suitability results (Figure 4).

Figure 4 Calibration Curve Results

Version:	v13	Calibration curve formula:	TOC: $y = 34.740x + 4.127$
Ver Creation:	2017/11/29 16:17	r ² value:	TOC: r ² = 0.99956
Comment:			
Operator:	Fusion (Fusion)		
Basic Analysis Type:	TOC		

Sample ID	Y Raw Value	X Expected	Message	End Time
0.100 ppm	7.6557	0.1000		2017/11/29 14:27
0.500 ppm	20.5680	0.5000		2017/11/29 14:51
1.000 ppm	36.8683	1.0000		2017/11/29 15:14
5.000 ppm	183.0770	5.0000		2017/11/29 15:38
10.000 ppm	349.1450	10.0000		2017/11/29 16:01

System Suitability Results

Bulk Water

Bulk water concentration is represented by 0.5 ppmC for both sucrose and 1,4-benzoquinone standards. Standards were prepared according to USP <643> guidelines. To pass <643> the reagent water must be less than 0.100 ppmC and the sucrose and 1,4-benzoquinone must have a response efficiency within 85-115% of each other using the equation shown in Figure 1. The response efficiency confirms how closely a difficult to oxidize compound and easy to oxidize compound respond in the system; ideally, it should be as close as possible. As shown in Figure 5 the Fusion TekLink software calculates and identifies if the standards have passed or failed.

Figure 5 Bulk Water Results which Easily Show Pass or Fail

Sample Type: System Suitability				
	Pos	System Suitability Sample Type	Sample ID	Result
◆	2	Reagent Water	[ReagentWater] 643 Sterile [Reagent Water]	0.0140 ppm (PASS)
◆	3	Standard Solution	[StandardSolution] 643 Sterile [Sucrose (500 ppb)]	0.5740 ppm
◆	4	Suitability Solution	[SuitabilitySolution] 643 Sterile [1,4-Benzoquinone (500 ppb)]	0.5614 ppm

Response Efficiency: 97.75%
(Acceptance Criteria 85% to 115%)

Formula
$\% \text{ Response Efficiency} = 100[(r_{ss} - r_w)/(r_s - r_w)]$
<ul style="list-style-type: none"> • r_{ss} = Instrument response to the System Suitability Solution (1, 4 Benzoquinone) • r_w = Instrument response to the Reagent Water Control • r_s = Instrument response to the Standard Solution (Sucrose)
Calculation
$\% \text{ Response Efficiency} = 100[(0.5614 - 0.0140) / (0.5740 - 0.0140)]$
Result
Response Efficiency = 97.75%
97.75% is within the 85%-115% required by USP <643>

Sterile Water

Sterile Water concentration is represented by 8.000 ppmC sucrose and 8 ppmC 1,4-benzoquinone standards. The standards were prepared according to USP <643>, which states that the Reagent Water must be less than 0.100 ppmC and the % Response Efficiency must be 85%-115%. The Sterile Water was run as a Check Standard and was within 15% of the KHP Standard.

The Fusion TekLink software calculates and identifies if the standards have passed or failed (Figure 6). The equation in Figure 1 was used to calculate the % Response Efficiency.

Figure 6 Bulk Water Standard Results

Sample Type: Check Standard --> Bulk

	Pos	BAT	Concentration (ppm)	Dil	Sample ID	Min / Max (%dev)	Result
◆	2	TOC	8.0000	1:1	[TOC] Bulk [8 ppmC Sucrose]	6.8000 / 9.2000 (85% / 115%)	8.1471 ppm (PASS)
◆	3	TOC	8.0000	1:1	[TOC] Bulk [8ppmC 1, 4B Benzoquinone]	6.8000 / 9.2000 (85% / 115%)	7.6145 ppm (PASS)

Formula
$\% \text{ Response Efficiency} = 100[(r_{ss} - r_w)/(r_s - r_w)]$
<ul style="list-style-type: none"> • r_{ss} = Instrument response to the System Suitability Solution (1, 4 Benzoquinone) • r_w = Instrument response to the Reagent Water Control • r_s = Instrument response to the Standard Solution (Sucrose)
Calculation
$\% \text{ Response Efficiency} = 100[(8.1471-0.0140) / (7.6145-0.0140)]$
Result
Response Efficiency = 107.01%
107.01% is within the 85-115% required by USP <643>.

Conclusion

The Teledyne Tekmar Fusion UV/Persulfate TOC Analyzer successfully ran USP Method <643> Bulk and Sterile Standard Concentrations using one calibration curve, saving valuable analysis time. The difficult to oxidize, 1,4- benzoquinone and easy to oxidize, sucrose, had a Response Efficiency of 85% - 115%, as required by USP <643>.

References

1. United States Pharmacopeia <643> Total Organic Carbon.[Online] <https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c643.pdf> (accessed 12/11/17)

See how the Teledyne Tekmar Fusion UV/Persulfate Analyzer can help you comply with pharmaceutical or environmental standards! Contact a sales representative at 1.800.874.2004 or visit <http://www.teledynetekmar.com/contact/sales-contacts>. See genuine customer reviews of the Fusion at <http://www.selectscience.net>.