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Analysis of Cleaning Validation Compounds using the TOC Fusion Analyzer

ABSTRACT

By taking an active approach to prevent cross-contamination in pharmaceutical and cosmetic production, international agencies such as the USFDA, ICH, EC, Health Canada, and the WHO have established regulations and guidelines for effective cleaning and sanitization.[1-6] With the potential side effects due to exposure, limiting carry-over of residual compounds into subsequent batches is crucial.

"Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."[7]

"Production equipment should be thoroughly cleaned on a scheduled basis."[8]

This study focuses on the analysis of cleaning validation samples using TOC without carryover concerns, negative impacts on precision or accuracy and the ease of developing a method for such analysis.

TOC IN PHARMACEUTICAL APPLICATION

One of the most widely used excipients is ultra-pure water and is also used in the manufacturing of drugs. Furthermore, it is also used for cleaning purposes. Different application areas therefore require different grades of pure water. The EP defines several grades of pure water quality: purified water, highly purified water, and water of injection. Purified water is used for the production of drugs that do not require a separate standard. The organic content is determined via the TOC value or the permanganate test. Highly purified water is sterile water for the production of drugs that do not require 'Water for Injection'. Water for injection is ultra-pure water that is used for the preparation of injection solutions. The TOC content of these different forms of pharmaceutical water may not exceed 0.5mg/L C per pharmacopoeia guidelines.

Analytical methods are validated to measure the active ingredient at levels consistent with its acceptable carryover into a subsequent batch of the next product. Most cleaning validation protocols are required to contain quantitative analytical determination of the analyte of interest and comparison of the analytical result to predetermined acceptance criteria. Biotech residues are typically analyzed using product specific immunological assays such as ELISA, or non-specific assays such as TOC. These protocol requirements are usually well defined in the cleaning validation master plan.[9]

Figure 1: The Fusion TOC Analyzer uses four amber Boston round bottles (125mL) in the built-in autosampler center position for calibration, calibration verification and system suitability analysis.

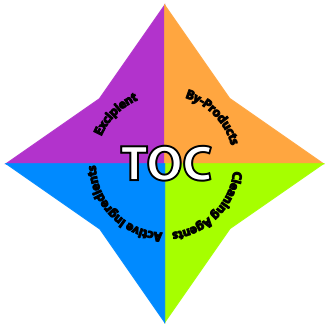

Figure 2: Since TOC measures carbon irrespective of its source, it can provide a system snapshot that is much more revealing than results from a compound-specific chromatogram. This simple illustration demonstrates how TOC can provide information about not simply the active ingredient in a manufacturing system, but also about cleaning agents, by-products, excipients and more.

Position 5 mg/L C	STD Concentration (mg/L C)	Fusion Dilution Factor	Result (Abs)	Std. Dev. (Abs)	RSD
C	0.0250	1:200	5.2773	0.1437	2.72%
C	0.0500	1:100	6.7143	0.0864	1.29%
C	0.1000	1:50	9.9673	0.1180	1.18%
C	0.5000	1:10	33.7270	0.0548	0.16%
C	1.0000	1:5	64.4600	0.3040	0.47%
C	5.0000	1:1	318.9633	2.7812	0.87%

Table 1: Calibration curve from 0.025mg/L C to 5mg/L C generated in triplicate using TekLink™ auto-dilution software feature from a single source standard.

RESULTS AND DISSCUSSION

Experimental

A calibration curve was generated using standard points ranging from 0.025 – 5.0mg/L of carbon using a single 5.0mg/L stock standard by employing the auto-dilution feature of TOC Fusion (Table 1). The default pharmaceutical method parameters of the TekLink™ software were selected. The default pre-installed methods are to aid the end user in method development and to accommodate multiple sample types.

There are two general types of sampling for cleaning validation. The USFDA states that the more desirable method is sampling of equipment surfaces using swabs or wipes. This direct method can be employed to physically remove contaminants.[10] Another common method is to sample rinse water after systems are cleaned per a validated cleaning procedure.[11] The use of rinse water analysis is necessary or preferable in those cases where access to surfaces using swabs is either impossible or impractical. The rinse water analysis involves flushing the cleaned system with purified water and obtaining samples of the final purified water flushes or “rinseate” sample for analysis.

The samples included in this study were prepared in a similar manner to analytical standards and simulate the simpler rinseate type sample, but are of the same basic design as swab samples for TOC analysis.[12] To ensure the robustness of the instrument’s oxidation processes, 1, 4-benzoquinone and sucrose were analyzed per USP 26 <643> compendial test for system suitability and response efficiency (Table 2). An E value of 98.31% was generated with this data, which is well within the USP specification of 85 – 115%.[13]

The TOC Fusion uses SPC detection technology, an advanced methodology for the measurement of TOC. SPC technology is a process by which a single measurement of the CO_2 inside a pressurized NDIR detector is taken. This is achieved by oxidizing the sample by either UV-Persulfate or HTC techniques. During the oxidation, the detector outlet is sealed allowing the CO_2 to be swept inside the detector to a predetermined pressure set-point. Once the pressure setting is achieved and all the CO_2 is pressurized inside the detector, a single CO_2 measurement is taken. The amount of CO_2 detected correlates to the amount of carbon in the sample.[14]

Position	System Suitability ID	Sample ID	Result	Std. Dev.	RSD
1	Reagent Water	[Reagent Water] USP 643 / EP 2.2.44 [Reagent Water]	0.0102 mg/L (PASS)	0.0004 mg/L	3.65%
2	Standard Solution	[Standard Solution] USP 643 / EP 2.2.44 [Sucrose (500 ppb)]	0.5113 mg/L	0.0022 mg/L	0.44%
3	Suitability Solution	[Suitability Solution] USP 643 / EP 2.2.44 [1,4-Benzoquinone (500 ppb)]	0.5028 mg/L	0.0029 mg/L	0.57%

Table 2: The purpose of the USP system suitability analysis is to test the ability of the TOC instrument to analyze a relatively easy to oxidize compound (sucrose) versus a relatively difficult to oxidize compound (1,4-benzoquinone). The precursor criterion is demonstration of low system water background (<0.100mg/L). The oxidation capability of the instrument is expressed as the Response Efficiency. In this instance it is 98.31%, which is the result of the suitability solution result divided by the standard solution result, expressed as a percent (Acceptance Criterion 85% to 115%).

Position	Chemical Formula	Molecular Mass	Sample ID	Percent Recovery	Result (ppm)	Std. Dev. (mg/L)	RSD
13	$C_{63}H_{88}CoN_{14}O_{14}P$	1355.37 g/mol	Vitamin B12	79.13%	0.7913	0.0232	2.93%
D	H_2O		Ultra Pure Water	N/A	0.0316	0.0021	6.77%
15	$C_5H_4NCO_2H$	123.11 g/mol	Vitamin B3	92.63%	0.9263	0.0157	1.70%
D	H_2O		Ultra Pure Water	N/A	0.0244	0.0042	7.14%
17	$C_5H_9NO_4$	147.13 g/mol	Glutamic acid	84.85%	0.8485	0.0131	1.55%
D	H_2O		Ultra Pure Water	N/A	0.0235	0.0035	15.03%
19	$C_{11}H_{12}N_2O_2$	204.23 g/mol	L-tryptophan	78.32%	0.7832	0.0102	1.30%
C	H2O		Ultra Pure Water	N/A	0.0226	0.0063	28.11%
21	N/A	52 kDa	Trypsin Inhibitor	85.55%	0.8550	0.0305	3.57%
C	H_2O		Ultra Pure Water	N/A	0.2168	0.0032	1.45%
23	N/A	150 kDa	Bovine IgG	29.01%	0.2901	0.0066	3.69%

Table 3: Summarizes results of compounds some of which are generally perceived as challenging to recover with UV-Persulfate TOC analysis based on their molecular structure and weight. Percent recoveries were in general good. The low recovery of Bovine IgG is probably due to its large molecular weight and uncharacterized structure. All recoveries are based upon a 100% theoretical concentration.

Compound Recovery

The robustness and versatility of using TOC in cleaning validation analyses has been questioned with respect to the robustness and versatility of the technique. The capability to use TOC analysis to effectively oxidize a compound is often gauged against the compound size or molecular weight. Table 3 summarizes results from a recovery study that included compounds which are generally perceived as challenging to recover with UV-Persulfate TOC analysis based on their molecular structure and weight. Percent recoveries ranged from 93% - 29%, with Vitamin B3 recovery being highest and Bovine IgG being lowest. The very low recovery of Bovine IgG is likely due to its very large structure, which has not been characterized for its carbon value.

Conclusion

Since Trypsin Inhibitor and Bovine IgG samples were not assayed to obtain the actual percent carbon, it is possible the recoveries are much better than reported. Bovine IgG is very likely greater than 50% carbon. As mentioned earlier, the default pharmaceutical method parameters were employed, optimizing the instrument for the best recovery for each compound would yield much higher recoveries. The ultra pure water samples analyzed in between each sample show very low carry-over of less than 0.50mg/L. The exception is less than 0.250mg/L seen after one of the larger proteinaceous compounds; however, well within most carryover specifications. The capability of the TOC Fusion analyzer to easily handle challenging compounds without carryover concerns and the use of the default pharmaceutical method parameters demonstrates the ease at which the TOC Fusion can be utilized for pharmaceutical applications.

REFERENCES

1. FDA, Guide to Inspections of Validation of Cleaning Process, 1993.
2. Health Canada, Drugs and Health Products, Cleaning Validation Guidelines (GUIDE-0028), January 10, 2008.
3. PIC/S, Pharmaceutical Inspection Convention, Recommendation on Validation Master Plan, Installation and Operational Qualification, Non-Sterile Process Validation, Cleaning Validation. July 2004.

ARTICLE ACRONYM LISTING

EC	European Commission
ELISA	Enzyme-Linked Immunosorbent Assay
EP	European Pharmacopeia
HTC	High Temperature Combustion
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
N/A	Not Available; large proteinaceous compound
NDIR	Non-Dispersive Infrared
ppb	parts per billion; equal to µg/L
mg/L	parts per million; equal to mg/L
SPC	Static Pressure Concentration
TOC	Total Organic Carbon
USFDA	United States Food and Drug Administration
USP	United States Pharmacopoeia
WHO	World Health Organization
ABS	Absorbance value