SHIMADZU

Application News

Total Organic Carbon Analysis No.041

Cleaning Validation by TOC Analyzer

To ensure quality control and safety in manufacturing facilities within the pharmaceutical industry, it is important that cleaning validation be conducted following the cleaning of production-related equipment. Cleaning validation ensures that the quantity of residual substances collected from the surfaces of the equipment is within permissible limit. Depending on the sampling method and measurement method used for this cleaning validation using a TOC analyzer, the following 3 types of methods are available.

(1) Rinse sampling – TOC measurement method
(2) Swab sampling – aqueous extraction – TOC measurement method
(3) Swab sampling – direct combustion carbon measurement method

Here we introduce the features of each of these methods, using the TOC-L$_{CPH}$ total organic carbon analyzer in the measurement of residual pharmaceutical products and their constituent substances.

■ Preparation of Residue Measurement Sample

In order to evaluate the cleaning validation sampling methods, residue measurement samples were created by applying various types of pharmaceutical products and their constituents to stainless steel pots. The aqueous and non-aqueous substances that were used are listed in Table 1. The aqueous substances and non-aqueous substances were dissolved in water and ethanol or acetone, respectively, and the solution concentrations were adjusted to 2000 mgC/L (= carbon concentration of 2000 mg/L). 100 µL of each solution was then applied to a 5 cm by 5 cm squares area on the surface of a stainless steel pot, and the respective solvents were dried out to produce residue measurement samples. Thus, the amount of carbon in the sample at each application site was 200 µg.
Among these, Gentacin ointment (aminoglycoside antibiotic) and Rinderon ointment (corticosteroid) were prepared based on determination of their carbon concentrations using the Shimadzu total organic analyzer system including the solid sample combustion unit.

Table 1 Sample Types

Substance Name	Solubility in Water	Solvent Used in Solution Preparation
Tranexamic acid	Soluble	Water
Anhydrous caffeine	Soluble	Water
Isopropylantipyrine	Insoluble	Ethanol
Nifedipine	Insoluble	Acetone
Gentacin ointment	Insoluble	Ethanol
Rinderon ointment	Insoluble	Acetone

■ (1) Rinse Sampling – TOC Measurement Method

The Rinse Sampling – TOC Measurement method is a technique in which the final rinse water used in the cleaning of a production equipment unit is used as the TOC measurement sample. This method is suitable for systems that cannot easily be disassembled, such as CIP (clean-in-place) equipment and narrow tubing. However, sampling is considered to be difficult if the residues are not soluble in water.

To evaluate the recovery of the various substances when using the Rinse Sampling – TOC Measurement method, 100 mL of pure water was transferred to the stainless steel pot with the patch of dried sample, and after stirring with a stirrer for 15 minutes to prepare the rinse solution, TOC measurement was conducted. Some of the measurement data are shown in Fig. 1.

Since the carbon content in each of the residue measurement samples is 200 µg, the TOC concentration would be 2 mgC/L if all of the sample were to dissolve in the water.

Now, for the blank, measurement was conducted in the same way using water that was transferred to the stainless steel pot, which in this case had no patch of dried sample applied to its surface. The measured blank concentration was subtracted from each TOC concentration, and then compared to the theoretical value of 2 mgC/L to determine the rate of recovery. The results are shown in Table 2. Water-soluble tranexamic acid and anhydrous caffeine had high recovery rates as expected. Moreover, water-insoluble isopropylantipyrine and nifedipine had high recovery rates. However, recovery rates of Gentacin ointment and Rinderon ointment were both low, at less than 20 %. From these results, it is clear that evaluation of the rinse water using this method is unreliable due to the variation of recovery of substances which are not readily soluble in water.

<Measurement Conditions>

Analyzer	: Shimadzu TOC-L$_{CPH}$ Total Organic Carbon Analyzer
Catalyst	: High sensitivity catalyst
Measurement item	: TOC (=TOC by acidification sparge processing)
Calibration curve	: 2-point calibration curve using 0-3 mgC/L potassium hydrogen phthalate aqueous solution
Injection volume	: 500 µL

Table 2 Measurement Results for Rinse Sampling – TOC Measurement Method

Substance Name	TOC Concentration [mgC/L]	Recovery Rate, [TOC Conc. – Blank / Theoretical Conc.]
Blank	0.030	-
Tranexamic acid	2.14	105 %
Anhydrous caffeine	2.19	108 %
Isopropylantipyrine	2.20	109 %
Nifedipine	2.17	107 %
Gentacin ointment	0.117	4.35 %
Rinderon ointment	0.333	15.2 %

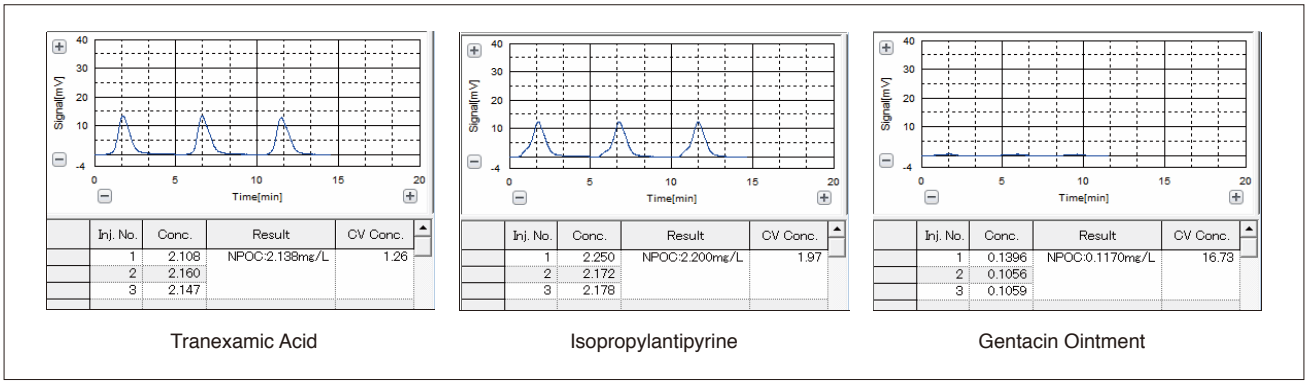

Fig. 1 Measurement Data Using Rinse Sampling – TOC Measurement Method

■ (2) Swab Sampling – Water Extraction – TOC Measurement Method

The Swab Sampling – Water Extraction – TOC Measurement method, as illustrated in Fig. 2, consists of wiping the inside surface of the production apparatus with a fibrous swab material, extracting the adhering material with water, and conducting TOC measurement of the extract solution. Since the residue is physically wiped off from a fixed area of the surface of the apparatus using the swab material, and then analyzed, the sampling efficiency is high. However, because water is used for extraction of the residue, residues that are insoluble in water are difficult to extract. Accordingly, cleaning evaluation with respect to these residues may be difficult for the same reason as that described with respect to difficult-to-dissolve substances in the (1) Rinse Sampling – TOC Measurement method.

To evaluate the recovery of the various substances when using the Swab Sampling – Water Extraction – TOC Measurement method, the sample, which was applied to a stainless steel pot, was wiped off with a 5 cm by 5 cm squares piece of fibrous swab material, which was then placed in a glass jar containing 100 mL of pure water. The residue was then extracted by stirring with a stirrer for 1 hour, after which TOC measurement was conducted. Some of the measurement data are shown in Fig. 3. Since the fibrous swab material (Alpha 10 obtained from Texwipe Co.) that was used is made of polyester, very little organic material is extracted from the swab itself. Since the carbon content in each of the residue measurement samples is 200 µg, the TOC concentration in the extraction solution would be 2 mgC/L if all of the sample were wiped off.

For the blank, measurement was conducted in the same way by wiping the stainless pot which had no sample applied before conducting extraction. The measured blank concentration was subtracted from each TOC concentration, and then compared to the theoretical value of 2 mgC/L to determine the rate of recovery. The results are shown in Table 3. Water-soluble tranexamic acid and anhydrous caffeine had high recovery rates as expected. Moreover, water-insoluble isopropylantipyrine and nifedipine had high recovery rates of about 90 %. However, recovery rates of Gentacin ointment and Rinderon ointment were both low, at less than 10 %. From these results, it is clear that evaluation of the rinse water using this method is unreliable due to the variation of recovery of substances which are not readily soluble in water.

<Measurement Conditions>

Analyzer	: Shimadzu TOC-L_{CPH} Total Organic Carbon Analyzer
Catalyst	: High sensitivity catalyst
Measurement item	: TOC (=TOC by acidification sparge processing)
Calibration curve	: 2-point calibration curve using 0 -3 mgC/L potassium hydrogen phthalate aqueous solution
Injection volume	: 500 µL
Swab material	: 5 cm by 5 cm squares piece of Texwipe Alpha 10 swab material washed in pure water and dried

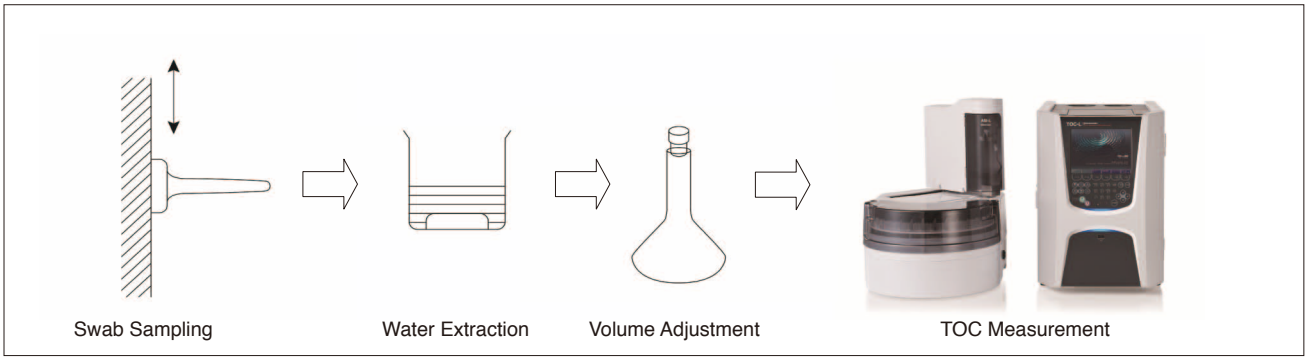

Fig. 2 Swab Sampling – Water Extraction – TOC Measurement Method

Table 3 Measurement Results for Swab Sampling – Water Extraction – TOC Measurement Method

Substance Name	TOC Concentration [mgC/L]	Recovery Rate, [TOC Conc. – Blank / Theoretical Conc.]
Blank	0.059	-
Tranexamic acid	2.19	107 %
Anhydrous caffeine	2.23	109 %
Isopropylantipyrine	1.90	92.2 %
Nifedipine	1.86	89.9 %
Gentacin ointment	0.093	1.70 %
Rinderon ointment	0.208	7.45 %

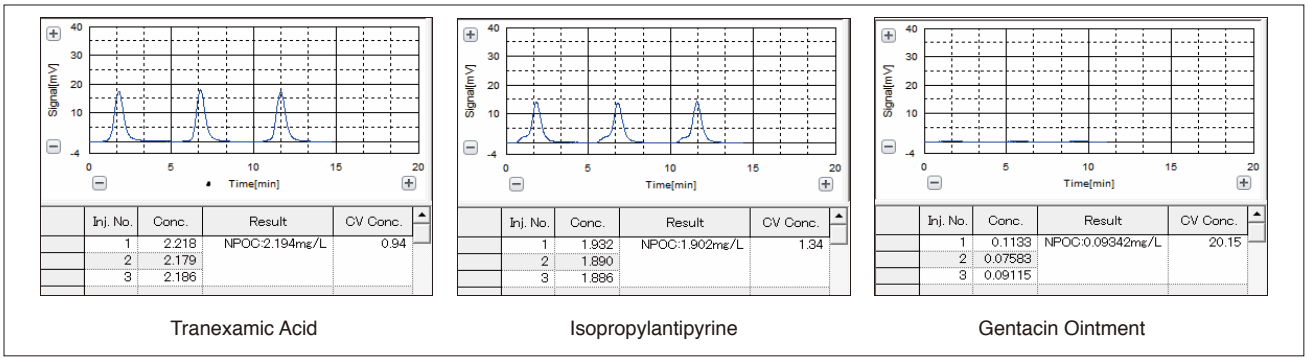

Fig. 3 Measurement Data Using Swab Sampling – Water Extraction – TOC Measurement Method

■ (3) Swab Sampling – Direct Combustion Method

The Swab Sampling – Direct Combustion method, as illustrated in Fig. 4, consists of wiping the inside surface of the production apparatus with a piece of inorganic quartz glass filter paper swab material, and then conducting measurement using a direct combustion carbon measurement system. The swab material with the adhering residue is merely placed in the sample boat, and the carbon content is measured directly by the TOC analyzer with the connected SSM-5000A Solid Sample Combustion Unit. By using this method, water-insoluble residues that are difficult to extract in water can also be collected, and measurement can be quickly and easily conducted without the need for any pretreatment, such as sample extraction, etc.

To evaluate the recovery rate of the different types of substances using the Swab Sampling – Direct Combustion method, we used the quartz glass filter paper swab material to wipe off the sample adhering to the stainless steel pot, placed the swab in the SSM-5000A sample boat, and conducted TC measurement. Some of the measurement data are shown in Fig. 5.

Since the carbon content in each of the residue measurement samples is 200 µg, the TC value would be 200 µg if all of the sample were wiped off. For the blank, measurement was conducted in the same way by wiping the stainless pot which had no sample applied. The measured blank value was subtracted from each TC value, and then compared to the theoretical value of 200 µg to determine the rate of recovery. The results are shown in Table 4. A high recovery rate of about 100 % was obtained for all the substances, regardless of whether they were water-soluble or water-insoluble.

<Measurement Conditions>

Analyzer	: Shimadzu TOC-L$_{CPH}$ Total Organic Carbon Analyzer + SSM-5000A Solid Sample Combustion Unit (IC circuit bypass using system with cell switching valve set)
Cell length	: Short cell
SSM carrier gas	: 400 mL/min oxygen gas
Measurement item	: TC
Calibration curve	: 1-point calibration curve using 30 µL of 1 % C glucose aqueous solution
Swab material	: Advantec QR-100 quartz glass filter paper (diameter 45 mm) heat-treated at 600 °C for 15 minutes

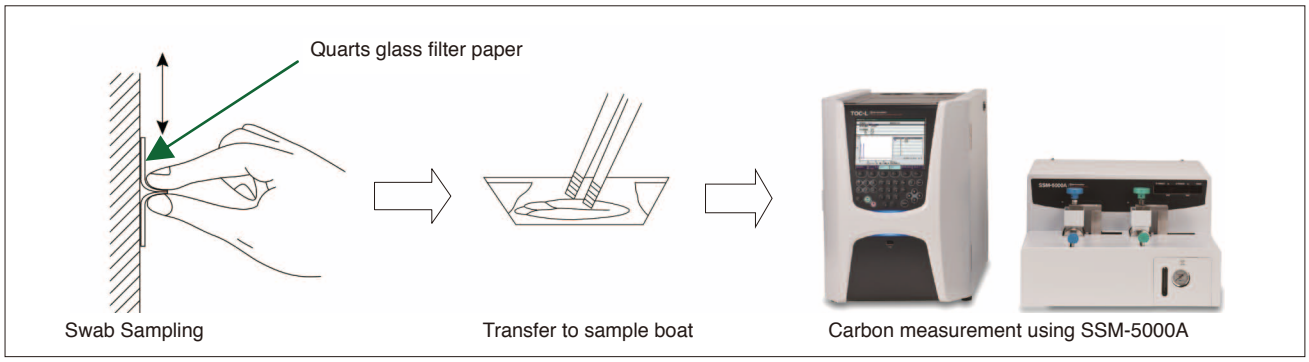

Fig. 4 Swab Sampling – Direct Combustion Method

Table 4 Measurement Results for Swab Sampling – Direct Combustion Method

Substance Name	TOC Value [µC]	Recovery Rate, [TC Value – Blank/Theoretical Value]
Blank	0.00	-
Tranexamic acid	202	101 %
Anhydrous caffeine	201	100 %
Isopropylantipyrine	210	105 %
Nifedipine	212	106 %
Gentacin ointment	200	100 %
Rinderon ointment	209	104 %

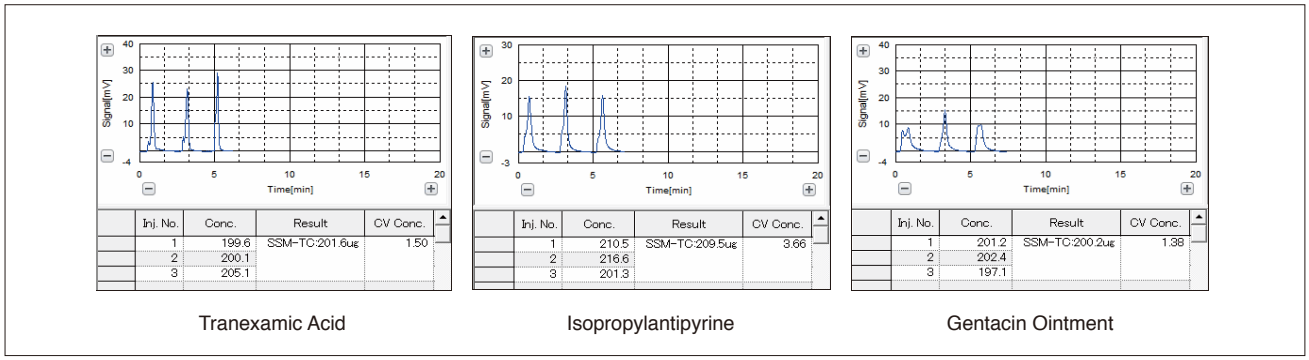

Fig. 5 Measurement Data Using Swab Sampling – Direct Combustion Method

■ Conclusion

The measurement methods used here and their respective recovery rates are summarized in Table 5.
When using the Rinse Sampling – TOC Measurement and the Swab Sampling – Water Extraction – TOC Measurement method, substances that do not easily dissolve in water were found to include those that had high recovery rates, and those that had low recovery rates. This may be due to differences in the strength with which the substances adhere to the stainless steel pot. Accordingly, it is probable that residue evaluation using these methods would be difficult for substances with low recovery rates.
In contrast to that, high recovery rates were obtained for all the substances when using the Swab Sampling – Direct Combustion method, regardless of whether the substances were water-soluble or water-insoluble, thereby permitting residue evaluation. Therefore, this method is considered to be an effective measurement method for conducting cleaning validation.

Table 5 Summary of Measurement Results

Substance Name	Solubility in Water	Recovery Rate		
		Rinse Sampling – TOC Measurement Method	Swab Sampling – WaterExtraction – TOC Measurement Method	Swab Sampling – Direct Combustion Method
Tranexamic acid	Soluble	105 %	107 %	101 %
Anhydrous caffeine	Soluble	108 %	109 %	100 %
Isopropylantipyrine	Insoluble	109 %	92.2 %	105 %
Nifedipine	Insoluble	107 %	89.9 %	106 %
Gentacin ointment	Insoluble	4.35%	1.70 %	100 %
Rinderon ointment	Insoluble	15.2%	7.45 %	104 %

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