GE
Water & Process Technologies
Analytical Instruments

Application Note

TOC Analysis of Compounds with Low Water Solubility; Evaluation of Swab Recoveries for Cleaning Validation Applications

The purpose of this study was to evaluate whether compounds with low solubility profiles could be recovered using Total Organic Carbon (TOC) analysis. In the Merck Index, *solubility profiles for these compounds are described as "substantially insoluble" or "practically insoluble." Our goal was to experimentally determine the solubilities of these compounds, and to investigate the percent recoveries from swabbing techniques. Due to confidentiality agreements, the identities of these compounds cannot be disclosed. Compounds A-F (see Table I) are small molecules (300-600 g/mol).*

Materials

- 12 x 12 cm stainless steel coupons, with a 10 x 10 etched area, washed with CIP-100, rinsed with low TOC water, and allowed to dry
- Powder-free gloves
- Volumetric flasks, cleaned according to Sievers procedure 914-80015
- Swabs (Texwipe Alpha Swab)
- Pre-cleaned 40 mL vials
- Volumetric pipette, 30 mL
- Hamilton gas-tight syringes, cleaned with CIP-100 and low TOC water
- Sievers* 800AS TOC Analyzer

Procedure

To minimize organic contamination, powder-free gloves were worn for the entire experiment. The solubility of each compound was determined empirically by adding the compounds to low-TOC water. The mixtures were shaken, stirred, and sonicated to help solubilize the compounds. After visual inspection, the carbon concentrations of the stock solutions were calculated as shown below.

$$\frac{\text{Mass of Compound (in mg)}}{\text{Volume (in L)}} \times \%\ \text{Carbon} = \text{ppm C}$$

Percent Carbon (% Carbon) is derived from the empirical formula for the compound.

$$\%\ \text{Carbon} = \frac{\text{Milligrams C}}{\text{Molecular Weight}}$$

For example, % Carbon for compound $C_{20}H_{22}N_4O_{10}S$ is:

$$\%\ \text{Carbon} = \frac{12 \times 20}{510.3} = 47\%\ \text{Carbon}$$

The carbon concentration of each stock solution was then verified using TOC analysis. The stock solutions of compounds A and B were analyzed directly, and for the stock solutions of compounds C through F, 10-fold dilutions were made. Prior to TOC analysis, a small aliquot (2 mL) of each stock solution was acidified with phosphoric acid to pH < 2. (In the case of solutions C through F, a small aliquot of the diluted solutions was acidified.) The resulting acidified solutions were visually inspected for evidence of precipitate formation. No precipitate was observed in any of the acidified solutions. The stock solutions A and B and the dilutions of the stock solutions C through F were then analyzed with a Sievers 800 TOC Analyzer.

The TOC results coincided with the calculated carbon concentrations, giving the following solubilities for the various compounds listed in Table I.

For the swab recovery study, the following solutions were prepared:

1. (2) vials of reagent water
2. (2) vials of background swab solution
3. (2) vials each of the spike solutions (12 total)
4. (2) vials each of swab recovery solutions (12 total)

Reagent Water: A 30 mL volumetric pipette was used to fill 28 pre-cleaned vials (40 mL) with 30 mL of low TOC water. After filling, each vial was immediately capped until further use. The (2) reagent water vials were labeled and set aside for subsequent TOC analysis. The remaining (26) filled vials were used to prepare the background swab solution, the spike solutions, and the swab recovery solutions.

Table 1. Results of Swab Recovery Studies

Reagent Water TOC	40 ppb
Background Swab TOC (3 swabs + water)	244 ppb

Cpd	Compound Class	Solubility in Water (23°C) (Stock Solutions) (ppm C)	Spike Solution TOC (ppm C)	Swab Recovery Solution TOC (ppm C)	Percent Recovery
A	steroid	17	0.577	0.773	99 %
B	b-lactam	25	0.821	0.976	94 %
C	sulfonamide	280	1.62	1.79	98 %
D	sulfonamide	150	1.03	1.20	97 %
E	pyrimidine	51	0.875	0.927	83 %
F	sulfonamide	50	1.05	1.26	100 %

Background Swab Solution: The two vials of background swab solution were prepared by cutting three swab tips into 30 mL of low TOC water. Care was taken to avoid contaminating the portion of the swab handle that was cut into the water.

Spike Solutions: The spike solutions (2 vials per compound) were prepared by spiking an aliquot of stock solution (aliquots ranged from 0.1-1.0 mL) into low TOC water (30 mL). For each compound, the selected aliquot made a final spike solution concentration of approximately 1 ppm C.

Swab Recovery Solutions: To prepare the swab recovery solutions, the same aliquot of stock solution used to prepare the spike solution was placed on to a stainless steel coupon. The solution was distributed evenly over the 10 x 10 cm coupon surface area and the coupon was allowed to dry (approximately 1 hour). Three swabs, pre-moistened with low-TOC water, were used in succession to swab the surface of the coupon. The three swabs tips were then cut into a vial of low TOC water (30 mL). All vials were shaken vigorously before analysis.

All vials (28) were analyzed using a Sievers Model 800AS, a TOC analyzer equipped with an autosampler. Analysis conditions were: Oxidizer flow rate of 0.2 mL/min, Acid flow rate of 0.75 mL/min. Four replicates were analyzed from each vial. The first replicate from each vial was disregarded and the last three replicates were averaged. Results from duplicate vials were then averaged to give the data shown in Table I. These data were used to calculate percent recoveries as shown in Figure 1.

Conclusions

Although compounds A through F are described in the *Merck Index* as "substantially insoluble" or "practically insoluble" in water, we have empirically determined that their solubilities at ambient temperature are in the part-per-million (ppm) range. These compounds were recovered successfully from stainless steel coupons using swabbing techniques and TOC analysis.

This study demonstrates the feasibility of using TOC for cleaning validation applications. Organic compounds, such as A through F, which are traditionally termed "insoluble" in water are indeed sufficiently soluble to be recovered using TOC analysis.

$$\textbf{Percent Recovery} = \frac{(\textbf{TOC}\ \text{Swab Recovery Solution} - \textbf{TOC}\ \text{Background Swab Solution})}{(\textbf{TOC}\ \text{Spike Solution} - \textbf{TOC}\ \text{Reagent Water})} \times 100\%$$

Example calculation (Compound A)

$$\text{Percent recovery} = \frac{(0.773\text{-}0.244) \times 100\%}{(0.577\text{-}0.040)} = 99\%$$

Figure 1. Calculation of Percent Recoveries from Swab Recovery Studies

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