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MICRO 90® CLEANING VALIDATION METHOD USING SWAB TECHNIQUE AND HPLC / MS

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Summary
A cleaning validation method has been developed for MICRO 90 Concentrated Cleaning Solution using a swab recovery technique and quantification with HPLC with charged aerosol detection (CAD) and mass spectrometry (MS) detection. The recovery of the sampling method was determined using HPLC-CAD; limit of detection (LOD) and limit of quantification (LOQ) were obtained using HPLC-MS.

The average recovery of triplicate samples was 95.1% with a standard deviation of 3.4%. Using a key sulfonate ingredient, the LOD and LOQ were 4.4µg/mL and 14.6µg/mL, respectively. Samples with concentrations of 2.27µg/mL and 4.45µg/mL were run as controls and obvious peaks of several ingredients were observed in the mass spectra, indicating that the LOD and LOQ calculations are very conservative. Using the swab technique for the surface residue check after a complete rinsing procedure, no peaks of the sulfonates were observed in the mass spectra, indicating a surface residue concentration less than 76 ng/cm^2.

Introduction
Surface rinsing and swabbing are two techniques used in cleaning validation. In this project, a cleaning validation of MICRO 90 was conducted with the surface swabbing technique. Combined with HPLC and MS detection methods, the limit of detection (LOD) and limit of quantification (LOQ) can be obtained and the recovery of the sampling method can also be evaluated. Customers may find this report helpful when developing their cleaning validation methods for MICRO 90.

Purpose:
The purpose of this research is to develop a cleaning validation method for MICRO 90 Concentrated Cleaning Solution using a swab technique and HPLC-MS instrumentation.

Experimental section:

1. Materials and reagents:

Sample:

a. MICRO 90® (Lot #140505);

Other chemicals:

a. Acetonitrile (Fluka; HPLC grade, 99.9%, Lot# SHBB6828V);
b. Deionized water (Millipore; 18.2 Ω);
c. Ammonium acetate (Sigma Aldrich; HPLC grade, 99.99%);
d. Acetic acid (Glacial, HPLC grade; Fisher Chemicals; Lot #112596);

Materials:

a. Q-Panels (Q-PANEL Lab Products, 2" x 4" 1008 Cold Rolled Steel)
b. Swabs (TX 714A Large Alpha® Swab, Texwipe® An ITW Company)

2. Sample preparation procedure:

   a. Standard solutions: All samples were filtered through syringe filters (13 mm × 0.45 µm, PVDF, General Separation Technologies, Inc.). MICRO 90® samples were serially diluted with a mixture of ACN and 0.1M pH 5.4 ammonium acetate buffer solution (1:1 v/v)).

   b. Recovery samples (triplicate): The same filtration procedure was followed to make a sample of MICRO 90 of 78.27 µg/µL. A 300 µL aliquot was transferred to a Q-panel surface of 58.06 cm^2. The panel was air dried at room temperature. After drying, a Texwipe pretreated with the buffer solution solvent was used to swab the Q-panel surface following the diagrams in Figure 1. After each step, the swab was swirled in the solution and pressed against the wall of a test tube. The three samples were sonicated for 20 min before filtration with syringe filters (13 mm × 0.45 µm, PVDF, General Separation Technologies, Inc.) for HPLC separation and CAD/MS detection.

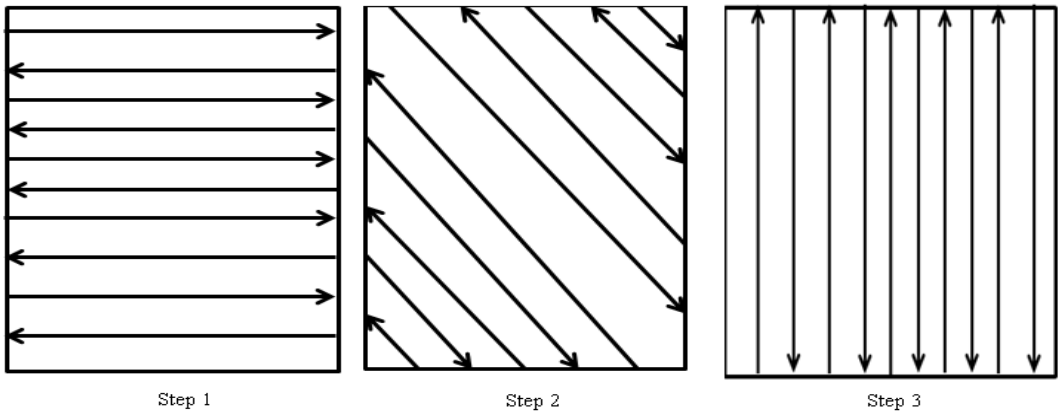

Figure 1 - Illustration of the swabbing procedure.

c. Residue test samples triplicate: A square Q-panel surface of 58.06 cm^2 was sprayed with a 2% MICRO 90 solution. Both sides of the Q-panel were subsequently rinsed under a faucet for 20 seconds and then sprayed with deionized water (Millipore; 18.2 Ω; 10 passes, and 5 times each side) using a Nalgene squeeze bottle. The Q-panel air dried at room temperature. After drying, a Texwipe pretreated with the buffer solution was used to sweep the Q-panel surface with force following the diagram in Figure 1. After each step, the swab was swirled in the solution and pressed against the wall of the test tube. The samples were sonicated for 20 min. The swabs were then discarded and the rest of the solution was evaporated to dryness with heating in a hood. The residue was diluted with 1 mL of a mixture of ACN and 0.1 M pH 5.4 ammonium acetate buffer solution (1:1 v/v) and sonicated for 10 min before filtration with syringe filters (13 mm × 0.45 µm, PVDF, General Separation Technologies, Inc.) for HPLC separation and MS detection.

Instrument: Dionex Ultimate 3000 HPLC equipped with a mass spectrometer (LCQ DECA XP ^{Plus}) as the detector

3. Data collection conditions:

A surfactant column (Acclaim® Surfactant Plus; dimensions: 3 µm 120 Å, 3 × 150 mm) was used for the separation. Mobile phase A was ACN; mobile phase B was 0.1M ammonium acetate + 5% ACN at pH 5.4. Each sample was injected by using an autosampler.
For the recovery calculation, CAD was used as the detector. A gradient elution was carried out from 35% of A and 65% of B. It was kept constant at this composition for 3 min. Mobil phase A was then increased to 80% and B was decreased to 20% in 15 min. It was kept constant at this composition for 8 min and then the system was restored to the start condition in 2 min. An equilibration time of 7 min was maintained for the system to restore its original condition. A total run was 35 min. The flow rate was 0.5 mL/min. The column temperature was controlled at 30 °C.

For the LOD and LOQ calculations, MS was used as the detector. A gradient elution was carried out using 50% A and 50% B. A was increased to 85% and B was decreased to 15% in 3 min. It was kept constant at this composition for 7 min and then the system was restored to the start condition in 2 min. An equilibration time of 7 min was maintained for the system to restore its original condition. The total run was 19 min. The flow rate was 0.5 mL/min. The column temperature was controlled at 30°C. For the MS detection, an ESI source was used with a spray voltage of 5 kV. The sheath gas flow rate was 60 and the auxiliary gas flow rate was 5. The source temperature was set to 250°C. The data was collected in the negative mode with scan range of *m/z* 180 to 700.

Results and Discussion:

1. Chromatograms of MICRO 90® with HPLC-CAD and MS under the negative mode

A sample of chromatograms by using the CAD and the MS (negative mode; *m/z* range: 180-700) of MICRO 90® are shown in Figures 2 and 3. Because the MS data was collected under the negative mode, only a few active ingredients showed signals in the mass spectra.

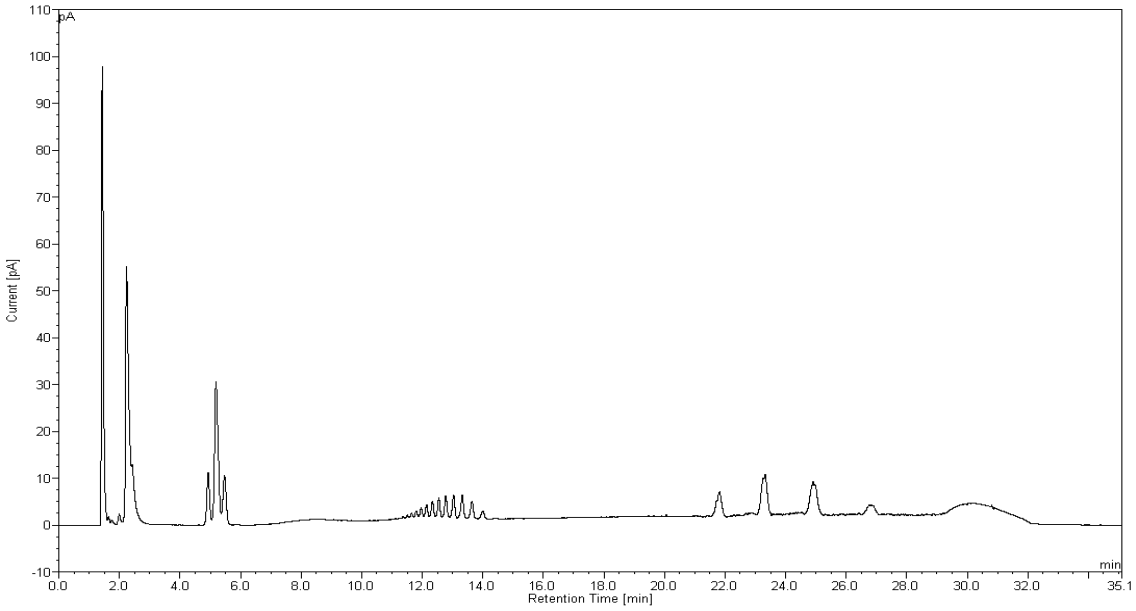

Figure 2. Chromatogram of a MICRO 90® sample with a CAD detector

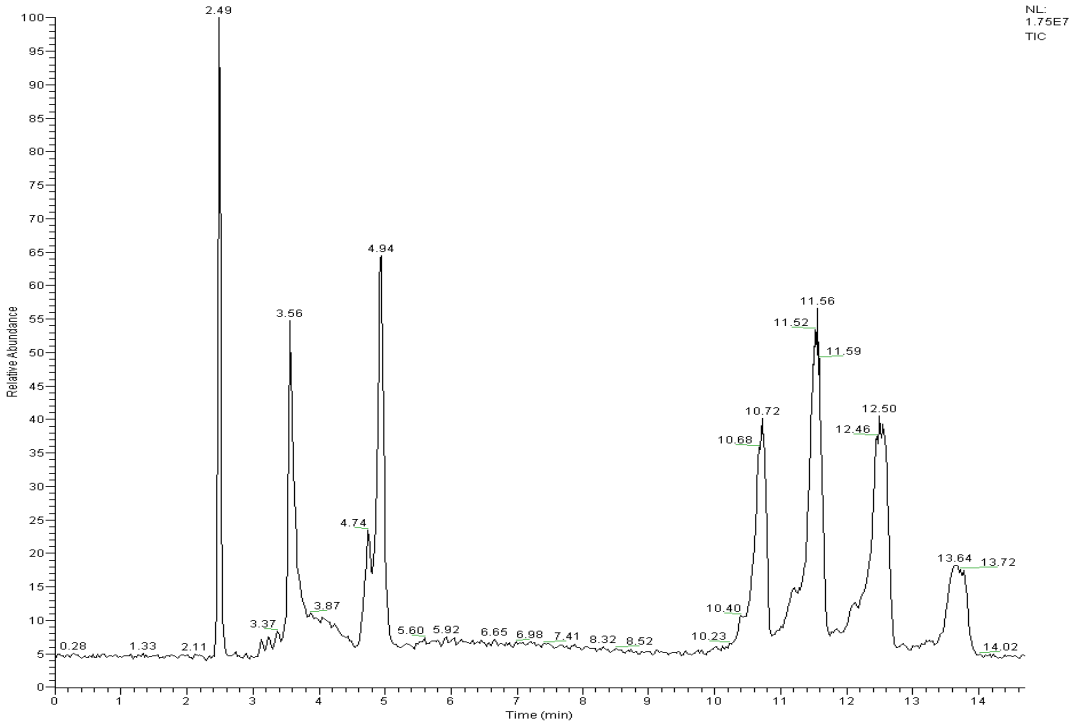

Figure 3 - Chromatogram of a MICRO 90® sample with a mass spectrometer detector in the negative mode.

2. Procedure for the determination of recovery of the sample

A standard curve was developed using HPLC-CAD for the recovery determination as seen in Figure 4. Using triplicate samples, the recovery was calculated and the values are shown in Table 1. The average recovery was 95.1% with a standard deviation of 3.5%. Filtration of the extracted swabbed samples with the PVDF syringe filters did not influence the recovery. A sonication time of 20 min was sufficient; the recovery did not increase with increased sonication time.

Table 1 – Recovery Data

	Detected amount (µg/uL)	Calculated amount (µg/uL)	Recovery (%)
Recovery - Sample 1	0.170	0.184	92.4
Recovery - Sample 2	0.173	0.184	94.0
Recovery - Sample 3	0.182	0.184	98.9

3. Determination of LOD and LOQ

Equations below were used in the calculation of LOD and LOQ:

LOD=3*(STEYX/SLOPE)

LOQ=10*(STEYX/SLOPE)

In which STEYX is the standard error of known response values Y and known concentrations X. The SLOPE is the slope of the calibration line of 6 samples with different concentrations. STEYX and SLOPE were obtained with Excel functions by using the data displayed in Table 2.

Table 2 – MICRO 90® samples' concentrations and peak intensities
With mass range of *m/z*=180-700

Concentration (µg/mL)	Intensity (mass range m/z 180-700)
44.54	2.96E+06
53.45	3.16E+06
76.97	3.76E+06
92.36	4.06E+06
110.83	4.58E+06
133.00	5.04E+06

These calculation methods of LOD and LOQ are more conservative than some other methods such as signal to noise ratios found by using the data explorer of mass spectrometer's work station software. The SLOPE was calculated to be 23756 by the Excel function as displayed in Figure 4. By plugging into the Excel spreadsheet, STEYX was calculated to be 34663. Therefore, the LOD and LOQ were calculated to be 4.4 and 14.6 ug/mL, which are similar to the previous results.

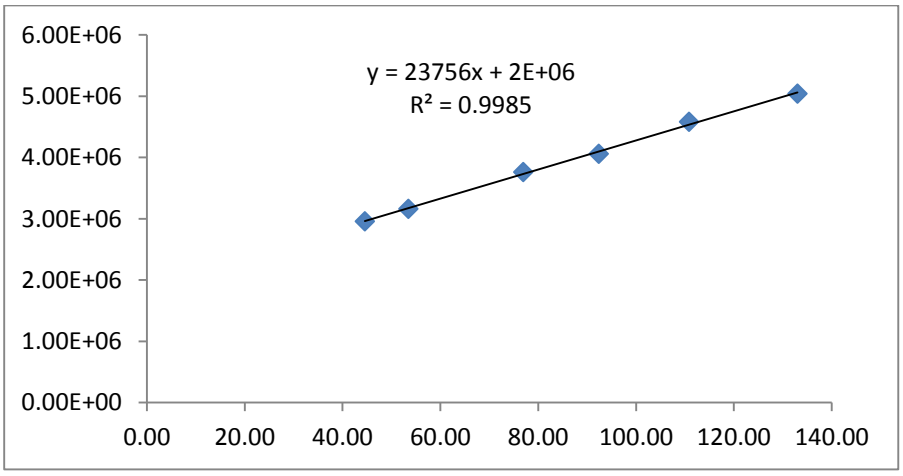

Figure 4 - Calibration line of MICRO 90® samples obtained by using an MS detector.

4. Validation of the LOD

Two validation samples (2.27 and 4.45 µg/mL) were detected by using this MS detection method along with all the other calibration samples, residue samples, and a solvent blank sample. For the solvent blank sample, a swab was extracted in 10 mL of the solvent for 20 minutes and then the extract was filtered before HPLC-MS detection. The mass spectra of all the validation samples, calibration samples, residue samples and the solvent blank were manually collected together as shown in Figure 5.

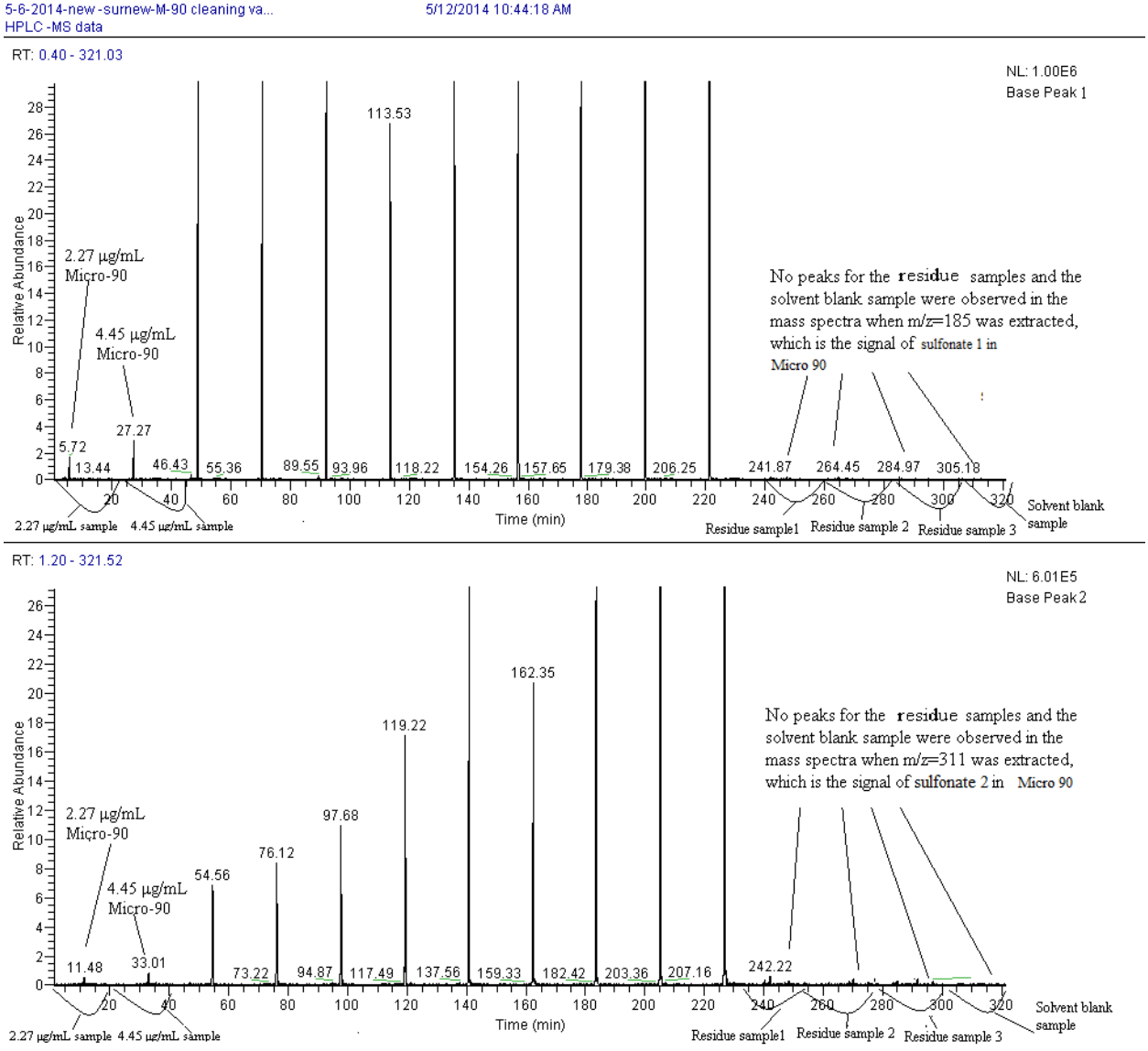

Figure 5. Mass spectra of MICRO 90: samples at concentrations of 2.27, 4,45 µg/mL, calibration samples, three residue samples and the solvent blank sample.

It can be seen that for the residue samples, no peaks of the extracted ions at *m/z* =185 and *m/z*=311 were observed, indicating the concentrations of the three samples were below the LOD. However, peaks of *m/z* 185 and 311 could be observed of MICRO 90 concentration of 2.27 and 4.45 µg/mL, which further proves that the obtained LOD was very conservative. In other words, if no peaks can be seen with the current method when *m/z* 185 and 311 are extracted in the negative ion mode, it is safe to say the concentration of MICRO 90 residue is below 76 ng/cm^2.

For more information and free product samples contact International Products Corporation:

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