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Development of a Cleaning Validation Method for MICRO-90® Using HPLC-CAD

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Abstract

A high performance liquid chromatography (HPLC) method with a Corona Charged Aerosol detector for the cleaning validation of MICRO-90® residue has been developed. The pooled average repeatability of 6 replicates at 6 concentration levels expressed as relative standard deviation (RSD) was 2.9% while the pooled average intermediate precision (intra-day repeatability in 4 days) of 6 replicates at 6 concentration levels was 3.0%. Simulated maximum allowable carryover (MAC) was calculated by using 10 ppm of MICRO-90® cleaner in a tablet of 1000 mg. With this MAC value, the amount of MICRO-90® cleaner applied to a stainless steel surface was calculated. The swabbed MICRO-90® samples were quantified by this HPLC-CAD method and the average recovery of 5 replicates of swabbed samples from stainless steel surfaces was about 79% ± 1%. The accuracy of the method was expressed as correlation coefficient, which was 99.63% by using all 3 sets of 6 replicates at 6 concentration levels in 4 days. The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 114 ng and 383 ng of MICRO-90®, respectively, by using the standard deviation of a matrix blank run. The value of LOD was verified by running a real sample. Specificity of the method was evaluated by conducting separation of the samples matrix, the recovery sample matrix, placebos (common drug excipients), and a mixture of placebos plus recovery sample matrix spiked with MICRO-90®. The result shows good resolution and absence of interference from the matrices and placebos. This method is designed for use in Food and Drug Administration (FDA) regulated establishments in compliance with Good Manufacturing Practices (GMP).

Keywords: Cleaning validation; MICRO-90®; Detergent; HPLC-CAD; Maximum allowable carryover, Good Manufacturing Practices

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1. Introduction

Residues of both drugs and cleaning agents can pose a serious problem if they accumulate over the maximum allowable carry over (MAC) level in final products. The Food and Drug Administration (FDA) described in its inspection guide that detergent residue must be removed after the cleaning process and stated proper analytical methods should be established with specified parameters such as method reliability, limits of detection (LOD) and quantification (LOQ), specificity, accuracy, etc.[1]. Many reports on cleaning validation of drug residues have been published in recent years [2-7]. However, reports related to cleaning validation of cleaning agent residues are not as common [8-10]. One of the reasons for this is that most cleaning agents are proprietary formulated products and the suppliers are not willing to disclose the ingredients; thus it is difficult for end users to develop a specific cleaning validation method accordingly without knowing the nature of some ingredient in a formulated cleaning agent. Various analytical analysis methods, such as high performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UPLC) [5], gas chromatography (GC) [11], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [12], atomic absorption spectroscopy (AAS)[13, 14] ion-mobility spectrometry (IMS)[15], total organic carbon (TOC) analysis [16-18], ultra-violet spectrophotometry (UVS)[7], etc., have been developed for cleaning validation and residue detection in the production area. Among all the analytical detection methods for cleaning validation, HPLC with all types of detectors remain the most popular method because of its convenience and availability of instruments.

In this work, a cleaning validation method was developed using HPLC equipped with a CAD detector. Ammonium xylene sulfonate (AXS) in MICRO-90® served as a probe in the detection of MICRO-90® residue. System repeatability, intermediate precision, accuracy, sensitivity, specificity, limit of detection (LOD) and limit of quantification (LOQ) were evaluated statistically. Because the maximum allowable carryover (MAC) value calculated by using the conservative LD_{50} value of MICRO-90® is much higher than using the conventional 10 ppm level, 10 ppm was used to calculate the MAC. MICRO-90® equivalent to the MAC level on a stainless steel surface was sampled by swabbing and the recovery was evaluated. Specificity of the method was also evaluated by comparing the chromatograms of the solvent matrix, the swabbed matrix and the placebos with that of a MICRO-90® sample. This work can be used by MICRO-90® end users in GMP industries that need to validate their cleaning procedure to ensure that the surface is free of MICRO-90® residue.

2. Materials and equipment

2.1. Equipment

A Dionex UltiMate® 3000 HPLC system equipped with a Corona charged aerosol detector was used for the separation. An Acclaim™ Surfactant Plus column (2.1 x 150 mm, 3 µm) was applied. A Cole-Parmer Instrument Company sonicator (8894) was used for the extraction of the swabbed samples.

2.2. Materials

MICRO-90® (Lot #2013-4-183) was used for the calibration curve development and swab sampling from surfaces. Acetonitrile (Fluka; HPLC grade, 99.9%), deionized water (Millipore; 18.2 Ω), ammonium acetate (Sigma Aldrich; HPLC grade, 99.99%) and acetic acid (Glacial, HPLC grade; Fisher Chemicals) were used to make the mobile phases and as solvent for MICRO-90® samples. Swabs (TX 714A Large Alpha® Swab, Texwipe® An ITW Company), stainless steel panels (10 cm x 10 cm) and 20 mL test tubes were used for swabbing recovery tests on surfaces. Syringe filters (13 mm × 0.45 µm, PVDF) purchased from General Separation Technologies, Inc. were used to filter all the samples prior to HPLC separation. Millipore® HVLP04700 Durapore® PVDF Membrane filter (0.45 µm) paper was used to filter all the solution in this experiment.

3. Experimental procedure

3.1. Preparation of mobile phases

Mobile phase A was made of 95% acetonitrile and 5% of Millipore deionized water. Mobile phase B was 0.1M pH 5.4 ammonium acetate buffer with 5% acetonitrile. All solutions were vacuum-filtered through 0.45 µm HVLP04700 Durapore® PVDF Membrane filter paper to remove any particulates that may have been present in the solutions. All the mobile phases were sonicated for 15 min at room temperature to degas the solutions before use.

3.2. Preparation of MICRO-90® stock solution and working standard solutions

The MICRO-90® stock solution was made by diluting 0.7848 g of MICRO-90® into a mobile phase mixture of 95%ACN+5% DI-water / 0.1M pH 5.4 ammonium acetate buffer solution+5% ACN

(1:1 v/v) and brought up to 50 mL. MICRO-90® samples were then diluted from the stock solution with the sample mobile phase mixture and the concentrations were 260.6 ng/µL, 470.9 ng/µL, 941.8 ng/µL, 1883.5 ng/µL, 1569.6 ng/µL and 2354.4 ng/µL. All standard samples were filtered through syringe filters prior to HPLC separation.

3.3. Calculation of MAC and concentration limit on production area

When the LD_{50} value was used for the MAC calculation, the calculation procedure is as follows.

Firstly, maximum daily allowed amount is calculated.

Maximum daily allowed amount =Safety factor X Body weight X LD_{50} value of MICRO-90®

Average body weight is assumed conservatively to be 60 kg (132.3 lb.). The LD_{50} value of MICRO-90® on its MSDS is >5 g/kg. Instead of using a usual safety factor of 0.1%, we will use 1 part per million.

Maximum daily allowed amount =1 ppm X 60 kg X 5 g/kg X 1000,000 µg/g=300 µg

The maximum allowed concentration in the next product can be calculated as follows.

Maximum allowed concentration=Maximum daily allowed amount / Maximum daily dose of a drug product

If a drug is prescribed at 4,000 mg/day, the maximum allowed concentration of MICRO-90® in the drug product can be calculated as:

Maximum allowed concentration = 300 µg /4 g=75 µg/g=75 ppm

Because this value is much higher than 10 ppm, 10 ppm is used as the maximum daily concentration of MICRO-90® in a drug product. Then MAC can be calculated using 10 ppm as the maximum daily concentration as follows:

MAC= Maximum daily concentration X Batch size of next product

If we assume the batch size is 100 kg, then MAC can be calculated as:

MAC=10 µg/g X 100 kg x 1000 g/kg=1000,000 µg

The amount allowed per surface area is calculated as follows.

Amount allowed per surface area =MAC / Production area

Let's assume the production area to be 25 m^2; then the amount per surface area will be:

Amount allowed per surface area = 1000,000 µg / (25 m^2 X 10000 cm^2 /m)= 4 µg/cm^2

If an area of 100 cm^2 is swabbed, the swabbed amount on this area should be:

Swabbed amount = Amount allowed per surface area X Swabbed area=4 µg/g X 100 cm^2 =400 µg

3.4. Preparation of the recovery samples

Five replicates of recovery samples were prepared by applying a 425 µL aliquot of a 941.8 µg/mL MICRO-90® sample (about 400 µg of MICRO-90®) to five stainless steel panels (10 cm x 10 cm), respectively. The panels were air dried at room temperature. After drying of the MICRO-90® sample on stainless steel surfaces, a Texwipe swab pretreated with 3 mL of a mobile phase mixture (A:B=1:1, v/v) in each test tube was used to wipe a panel surface following the diagrams in Figure 1. After each step, the swab was inserted in the extracting solution in the test tube. A dry swab was then used to wipe the surface by following the steps and was also placed into the extracting solution. The five samples and a matrix blank with two swabs in each sample were sonicated for 15 min and the swabs were pressed against the inner wall of the test tubes. Afterwards, the solution volume was a little less than 2 mL. Each of the recovery samples was brought up to 2 mL by adding the 1:1 mobile phase mixture. All the recovery samples were filtered through 0.45 µm PVDF syringe filters before separation with HPLC.

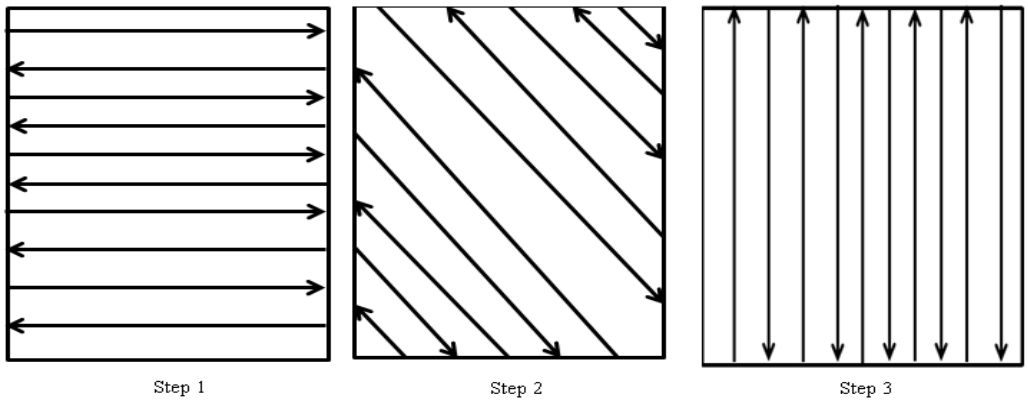

Figure 1 - Illustration of the swabbing procedure.

3.5. Preparation of specificity samples

A placebo sample was made by using some common excipients in pharmaceuticals as shown in Table 1. A sample matrix (a mobile phase 1:1 mixture), a placebo sample, a recovery matrix blank, and a mixture of placebo with the recovery matrix blank spiked with MICRO-90® were prepared for the specificity evaluation.

Table 1. Composition of the placebo sample

Name	Weight (g)
Polyethylene glycol 8000 powder	0.0178
Magnesium stearate	0.0038
Crospovidone	0.0075
Microcrystalline cellulose	0.0049
Polyethylene glycol 400 (Carbowax Sentry™ 400)	0.085
Corn starch 400 L	0.016
Soybean oil	0.0189
Wax	0.0143

3.6. HPLC separation conditions

A surfactant column (Acclaim® Surfactant Plus; dimensions: 3 µm, 2.1 × 150 mm) was used for the separation. Mobile phase A was 95% acetonitrile + 5% of Millipore deionized water; mobile phase B was 0.1M ammonium acetate + 5% ACN at pH 5.4. Each sample was injected 5 µL by using an autosampler.

A gradient elution was carried out from 15% of A and 85% of B. It was kept constant at this composition for 6 min. Mobil phase A was then increased to 80% and B was decreased to 20% in 10 min. It was kept constant at this composition for 4 min and then the system was restored to the start condition in 5 min. An equilibration time of 7 min was maintained for the system to restore its original condition. A total run was 32 min. The flow rate was 0.300 mL/min. The column temperature was controlled at 23°C.

4. Results and discussion

4.1. Repeatability and intermediate precision

The HPLC system repeatability was evaluated by injecting 6 replicates at 6 concentration levels of each sample. The intermediate precision was evaluated by repeatedly injecting 3 sets of the 6

replications at 6 concentration levels. Both repeatability and intermediate precision were obtained by using area responses and analysis of variance (ANOVA). The average repeatability of all 6 concentrations is 2.9% expressed as the pooled relative standard deviation of all 36 injections within 24 hours. The intermediate precision of 3.0% was obtained by pooling the relative standard deviations of these three sets of injections in 4 days.

4.2. Specificity

The method specificity was evaluated by comparing chromatograms of the sample matrix (the 1:1 mobile phase mixture), the placebo (a common excipients mixture), a mixture of placebo and recovery sample matrix blank spiked with MICRO-90® . The overlaid chromatograms are shown in Figure 2. It can be seen that there is no interference to the AXS peaks from any matrices.

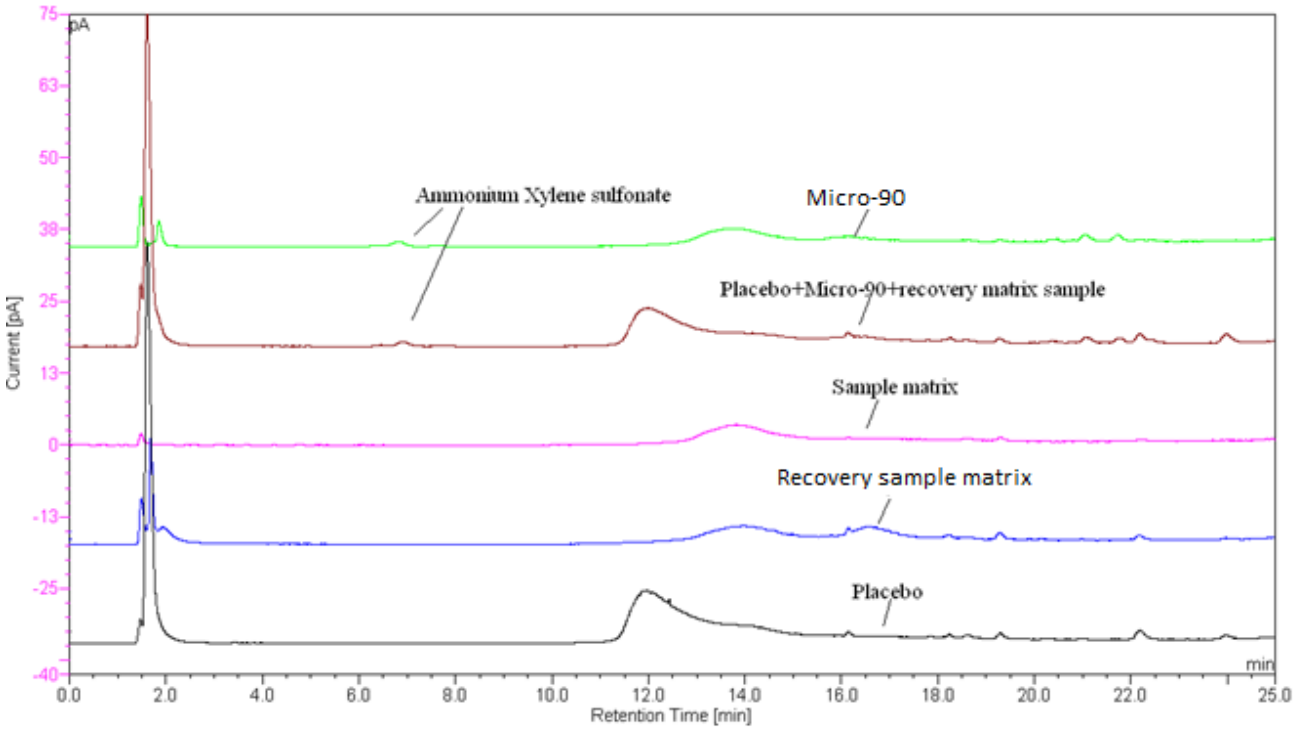

Figure 2. Comparison of chromatograms of MICRO-90®, a sample matrix (the 1:1 mobile phase mixture), a recovery sample matrix, a placebo (a common excipients mixture), and a mixture of placebo plus recovery sample matrix spiked with MICRO-90®.

4.3. Accuracy/Recovery

The accuracy of this method was evaluated by correlation coefficient of the MICRO-90® standard quadratic curve fitting. A correlation coefficient R^2 of 99.63% was obtained from all 3 sets of 6 replicates at 6 concentration levels (108 runs total) by using area responses. The dynamic range of a CAD detector is from low ng level to high µg level.

Five replicates of the swabbed samples were quantified by using the standard curve and the chromatograms are shown in Figure 3. Great consistency in the composition of the recovery samples is seen in the chromatograms. The average recovery of the 5 replicates of the swabbed samples was 79%± 1%, which was fairly good when the low concentration level of swabbed samples is taken into consideration.

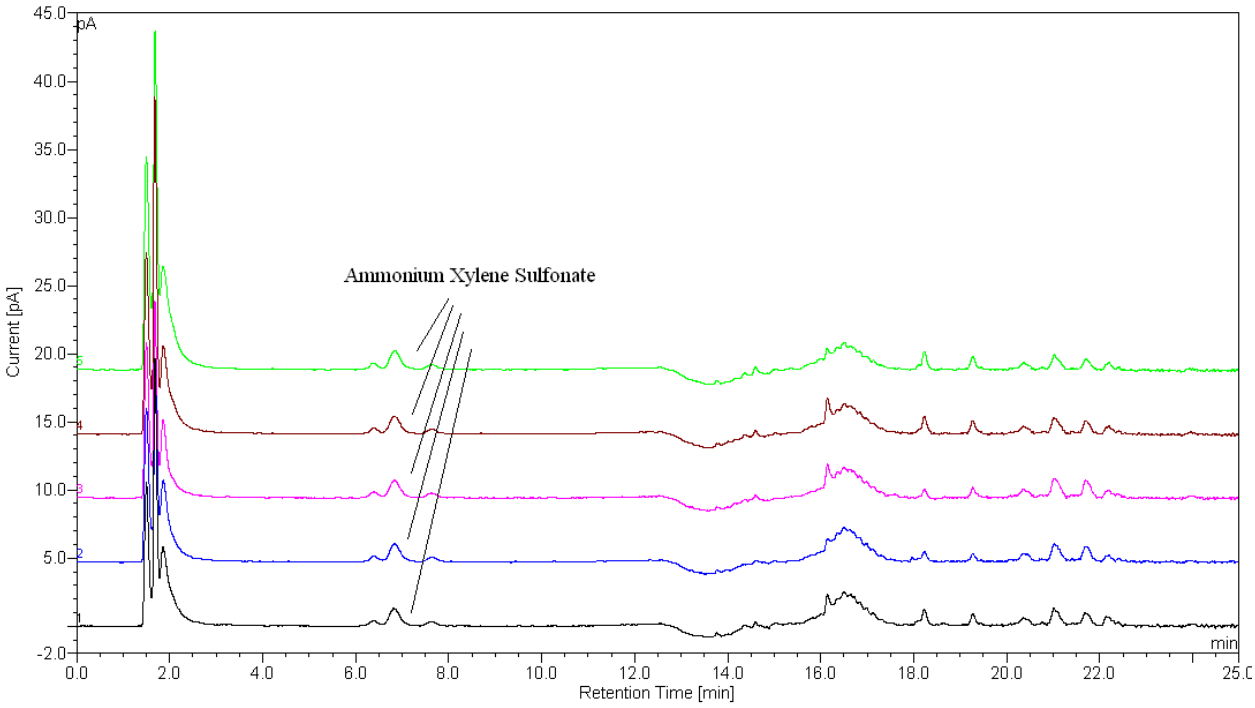

Figure 3. Chromatograms of 5 recovery sample replicates.

4.4. Sensitivity

The limit of detection (LOD) of 114 ng was calculated by using 3 times of the standard deviation of the noise level in a matrix blank. The limit of quantitation (LOQ, 10 times of the standard deviation of the blank noise level) was calculated to be 383 ng.

5. Conclusion

From all the results obtained in this work, it is safe to claim that an HPLC-CAD system is suitable for cleaning validation of MICRO-90®. This system and method developed are sensitive, accurate and easy to use. The method developed can be used as an example for GMP industries to develop their own protocols for cleaning validation of MICRO-90® residue and other similar cleaning agent residues. The calculation procedure is especially useful as a template for analysts to follow in their real world calculation.

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