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Development of a Cleaning Validation Method for MICRO® A07 Citric Acid Cleaner 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® A07 Citric Acid Cleaner residue has been developed. The pooled average repeatability of 3 replicates at 6 concentration levels expressed as RSD was 1.3 % while the pooled average intermediate precision (intra-day repeatability in 3 days) of 3 replicates at 6 concentration levels was 2.4 %. Simulated maximum allowable carryover (MAC) was calculated by using 10 ppm of MICRO® A07 Citric Acid Cleaner in a tablet of 1000 mg. With this MAC value, the amount of MICRO® A07 Citric Acid Cleaner applied to a stainless steel surface was calculated. The swabbed MICRO® A07 Citric Acid Cleaner samples were quantified by this HPLC-CAD method and the average recovery of 5 replicates of swabbed samples from stainless steel surfaces was about 92 % ± 2%. The accuracy of the method was expressed as correlation coefficient, which was 99.7% by using all 3 sets of 3 replicates at 6 concentration levels in 3 days. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 60 ng and 189 ng, respectively, by using the three times and ten times 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 blanks, the samples matrix, and placebos (common drug excipients) plus recovery sample mixture spiked with MICRO® A07 Citric Acid Cleaner. The result shows good resolution and absence of interference to the targeted peaks from the matrices and placebos. This method is designed for use in Food and Drug Administration (FDA) regulated establishments in compliance with good manufacturing practice (GMP).

Keywords: Cleaning validation; MICRO® A07 Citric Acid Cleaner; Detergent; HPLC-CAD; Maximum allowable carryover, Good manufacturing practice

1. Introduction

Residues of both drugs and cleaning agents can pose a serious problem if they accumulate 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, 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 in lack of cleaning validation of cleaning agent residues is that most cleaning agents are proprietary formulated products and the suppliers are not willing to disclose the ingredients in their formulas; thus it is difficult for the end users to develop a specific cleaning validation method accordingly without knowing the nature of the 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. Citric acid in MICRO® A07 Citric Acid Cleaner served as a probe in the detection of MICRO® A07 Citric Acid Cleaner 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® A07 Citric Acid Cleaner is much higher than using the conventional 10 ppm level, 10 ppm was used to calculate the MAC. MICRO® A07 Citric Acid Cleaner 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® A07 Citric Acid Cleaner sample. This work can be used by MICRO® A07 Citric Acid Cleaner end users in GMP industries who will need to validate their cleaning procedure to ensure the surface is free of MICRO® A07 Citric Acid Cleaner residue.

2. Materials and equipment

2.1. Equipment

Dionex Ultimate 3000 HPLC equipped with a Corona charged aerosol detector was used for the separation. An ACE SuperC18 column (2.1 x 250 mm, 3 µm, Advanced Chromatography Technologies Ltd.) was applied. A Cole-Parmer Sonicator (8894) was used for the extraction of the swabbed samples.

2.2. Materials

MICRO® A07 Citric Acid Cleaner (Lot #150608) 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%), formic acid (GFS, HPLC grade) and acetic acid (Glacial, HPLC grade; Fisher Chemicals) were used to make the mobile phases and as solvent for MICRO® A07 Citric Acid Cleaner 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 3% acetonitrile and 97% of Millipore deionized water with 0.1% of formic acid. Mobile phase B was 20% of a 0.1 M pH 5.4 ammonium acetate buffer plus 80% acetonitrile. All aqueous solutions were vacuum-filtered through 0.45 µm HVLP04700 Durapore® PVDF Membrane filter paper to remove any particulates that may be 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® A07 Citric Acid Cleaner stock solution and working standard solutions

The MICRO® A07 Citric Acid Cleaner stock solution was made by diluting MICRO® A07 Citric Acid Cleaner into the mobile phase A. MICRO® A07 Citric Acid Cleaner samples were then diluted from the stock solution with the mobile phase A and the concentrations were 282.0 ng/µL, 310.2 ng/µL, 338.4 ng/µL, 366.6 ng/µL, 394.8ng/µL and 423.0 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® A07 Citric Acid Cleaner

Average body weight is assumed conservatively to be 60 kg (132.3 lb.). The LD_{50} value of MICRO® A07 Citric Acid Cleaner on its MSDS is >5 g/kg. Instead of using a usual safety factor of 0.1%, we will use 1 parts per million.

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

Maximum allowed concentration in 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® A07 Citric Acid Cleaner 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® A07 Citric Acid Cleaner 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 1289 µL aliquot of a 310.2 µg/mL MICRO® A07 Citric Acid Cleaner sample (about 400 µg of MICRO® A07 Citric Acid Cleaner) to five stainless steel panels (10 cm x 10 cm), respectively. The panels were air dried at room temperature. After drying of the MICRO® A07 Citric Acid Cleaner sample on stainless steel surfaces, a Texwipe swab pretreated with 3 mL of the mobile phase A in each 20 mL centrifuge vial was used to wipe a panel surface following the diagrams in Figure 1. After this step, the swab was inserted in the extracting solution in the vial. 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 with lid on to avoid evaporation of solvent. The swabs were taken out upon finishing. Although the resulting solution was slightly less than 3 mL, the calculated concentration of A07 in the recovery solution was maintained as the same if the recovery rate was 100%, which was 400 ug/3 mL (133.3 ng/µg). No solvent was lost by evaporation. Instead, it was only

re-distributed between the vial and the swabs. All the recovery samples were filtered through 0.45 µm PVDF syringe filters before separation with HPLC.

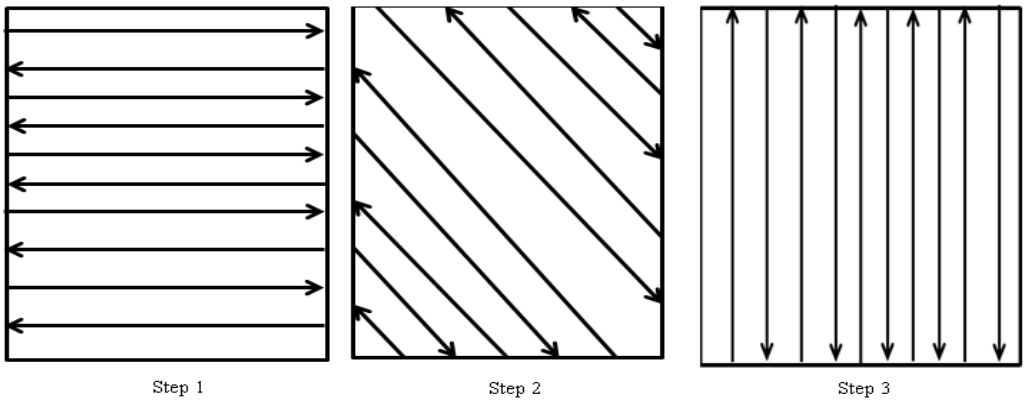

Figure 1 - Illustration of the swabbing procedure.

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 recovery matrix blank, and a mixture of placebo with the recovery matrix blank spiked with MICRO® A07 Citric Acid Cleaner were prepared for the specificity evaluation.

Table 1. Composition of the placebo sample*

Name	Weight (g)
Polyethylene glycol 8000 powder	0.0228
Magnesium stearate	0.0252
Crospovidone	0.0242
Microcrystalline cellulose	0.0269
Polyethylene glycol 400 (Carbowax Sentry™ 400)	0.0267
Corn starch 400 L	0.0245
Soybean oil	0.0208
Wax	0.0309

* Diluted with 10 mL of a 1:1 mixture of HPLC mobile phases.

3.6. HPLC separation conditions

An ACE Super C18 column (Advanced Chromatography Technologies Ltd; dimensions: 3 µm, 2.1 × 250 mm) was used for the separation. Mobile phase A was 97% of Millipore deionized water with 0.1% of formic acid. Mobile phase B was 20% of a 0.1 M pH 5.4 ammonium acetate buffer plus 80% acetonitrile. Each sample was injected 4 µL by using an autosampler.

A gradient elution was carried out from 100% of B. It was kept constant at this composition for 4 min. Mobile phase A was then increased to 100% in 5 min. It was kept constant at this composition for 10 min and then the system was restored to the start condition in 3 min. An equilibration time of 10 min was maintained for the system to restore its original condition. A total run was 32 min. The flow rate was 0.250 mL/min. The column temperature was controlled at 40 °C.

4. Results and discussion

4.1. System repeatability and intermediate precision

The HPLC system repeatability was evaluated by injecting 3 replicates at 6 concentration levels of each sample. The intermediate precision was evaluated by repeatedly injecting 3 sets of the 3 replications at 6 concentration levels. Both system repeatability and intermediate precision were obtained by using area responses and analysis of variance (ANOVA). The average system repeatability of all 6 concentrations is 1.3% expressed as the pooled relative standard deviation of all 18 injections within 24 hours. The intermediate precision of 2.4% was obtained by pooling the relative standard deviations of these three sets of injections in 3 days.

4.2. Specificity

The method specificity was evaluated by comparing chromatograms of the sample matrix, the recovery sample matrix, and the swabbed recovery sample matrix plus a placebo (a common excipients mixture) spiked with MICRO® A07 Citric Acid Cleaner. A chromatogram of a recovery sample matrix plus a placebo spiked with MICRO® A07 Citric Acid Cleaner is shown in Figure 2. It can be seen that there is no interference to the citric acid peak from any matrices.

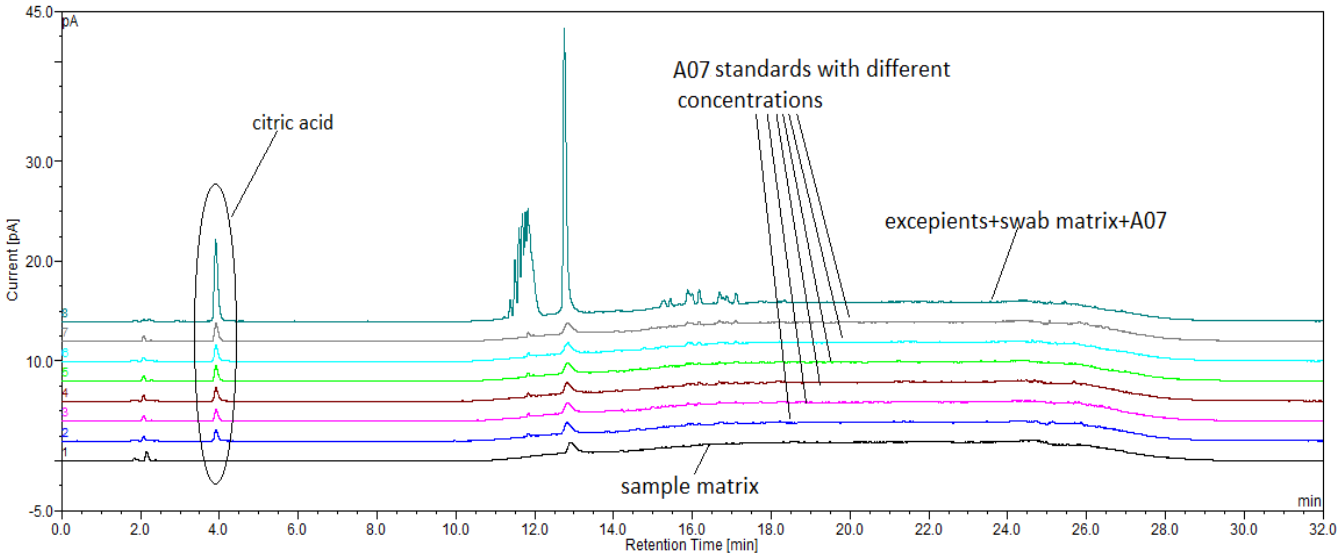

Figure 2. Chromatograms of a sampler matrix, 6 standards and a swabbed matrix plus an excipient placebo spiked with MICRO® A07 Citric Acid Cleaner.

4.3. Accuracy/Recovery

The accuracy of this method was evaluated by correlation coefficient of the MICRO® A07 Citric Acid Cleaner standard quadratic curve fitting. A correlation coefficient R^2 of 99.7% was obtained from all 3 sets of 3 replicates at 6 concentration levels (54 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 92%± 2%.

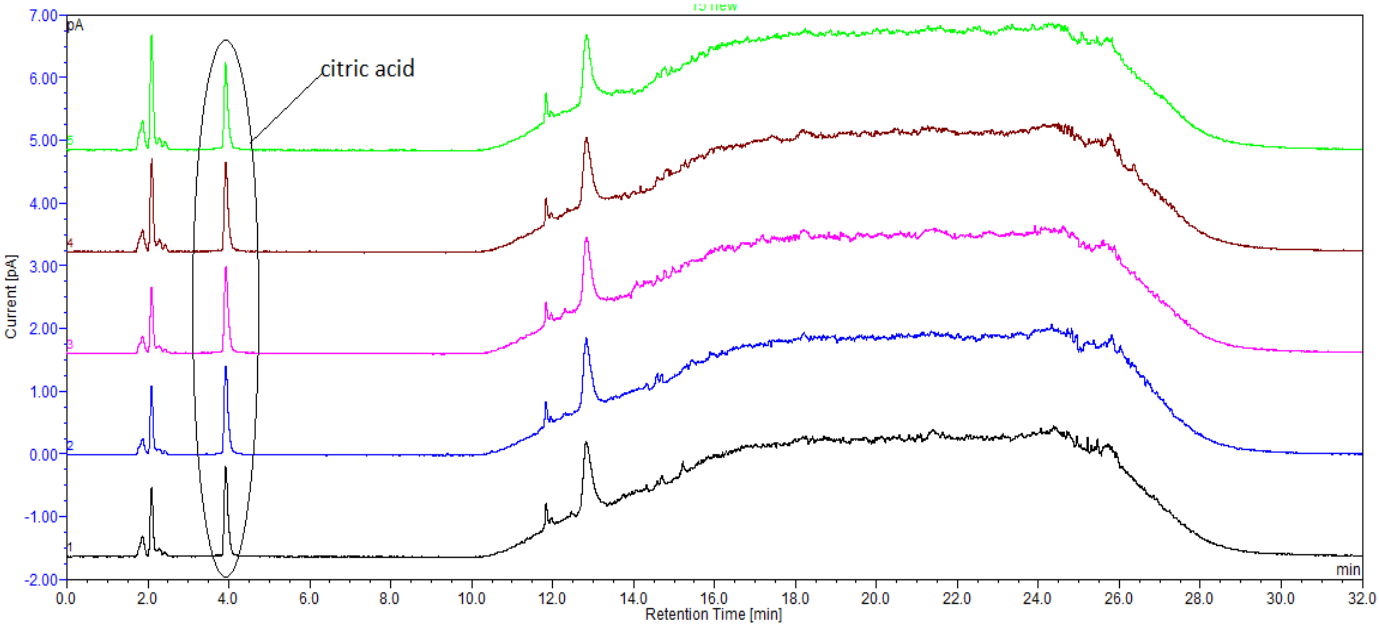

Figure 3. Chromatograms of 5 recovery sample replicates.

4.4. Sensitivity

The limit of detection (LOD) of 60 ng was calculated by using 3 times of the standard deviation of a matrix background. The limit of quantitation (LOQ) was calculated to be 189 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® A07 Citric Acid Cleaner in pharmaceutical industries and any other environments that would require cleaning validation of cleaning agent residues. 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® A07 Citric Acid Cleaner 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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