



Rethinking Cleaning Validation for API Manufacturing

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Cleaning validation is a regulatory requirement worldwide and an integral process step within the manufacturing of active pharmaceutical ingredients (APIs) and drug products. The approach of designing, qualifying, and monitoring a process is labeled a lifecycle approach. This article focuses on applying new and traditional techniques to design a cleaning process, ensure the surfaces are clean, and develop rinse solution analysis to continuously monitor cleaning performance.

Cleaning validation is a regulatory requirement worldwide and an integral step within the manufacturing of active pharmaceutical ingredients (APIs), pharmaceuticals, and biopharmaceutical products. Manufacturers strive to improve the efficiency, cost, quality, and time required to design, qualify, and monitor the cleaning process. This approach of designing, qualifying, and monitoring a process, such as cleaning, is referred to as the lifecycle approach (1–2). The goal of a successful cleaning validation program is that cleaning procedures are defined, analytical methods are qualified, and acceptance criteria are continuously met.

A laboratory cleaning evaluation was performed on 15 generic APIs (3). The cleaning performance results combined with solubility and toxicity information were used to select three APIs. The Fourier-transform infrared spectroscopy (FTIR) direct-surface sampling technique combined with visual inspection were used to ensure the product contact surface is clean. The surface sampling used during the cleaning validation runs was combined with ultraviolet spectroscopy (UV) of a rinse solution. The UV monitoring can be used in-line to continuously monitor the API content in the final rinse solution (4–5). In-line monitoring of the rinse solution can also be used to monitor removal of cleaning agents. Designing a risk-based cleaning process correlating direct-surface sampling, visual inspection, and coverage testing to in-line rinse solution enables an efficient, low-cost means to continuously monitor the API equipment cleaning procedure (6).

The aim of this study is to demonstrate that swabbing and swab analysis for detergents may in some cases be replaced with direct and in-line methods, which offer improved efficiency for API manufacturers. To achieve this aim, the authors adopted a cleaning protocol that eliminates the need for swabbing and swab analysis during routine monitoring by using direct FTIR spectroscopy surface testing, while the entire process is continuously monitored in-line by UV spectroscopy.

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Table I: API description, formula, molecular mass, structure, and solubility. LOD is limit of detection. LD₅₀ is the lethal dose of active that kills 50% of a sample population.

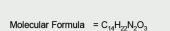
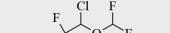
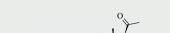
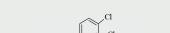
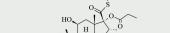
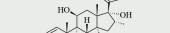
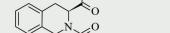
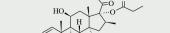
Actives* (chemical abstracts registry number)	General indication*	Structure, formula, and molecular mass*	Spectral properties	Solubility*	Toxicity (LD ₅₀ rat)
Atenolol [29122-68-7]	Anti-hypertensive, anti-anginal, anti-arrhythmic	 Molecular Formula = C ₁₄ H ₂₂ N ₂ O ₃ Formula Weight = 266.34	UV max absorption at 226 nm (7), LOD 0.5 µg/mL	Slightly soluble in water	Oral LD ₅₀ rat 3000 mg/kg IV LD ₅₀ rat 59.24 mg/kg (7) Oral LD ₅₀ rat >2000 mg/kg IV LD ₅₀ rat 77 mg/kg (8)
Gabapentin [60142-96-3]	Anti-convulsant; analgesic	 Molecular Formula = C ₉ H ₁₃ NO ₂ Formula Weight = 171.24	UV max absorption at 210 nm, Linear range: 0.25-3.5 µg/mL.	Freely soluble in water	Oral LD ₅₀ rat >8000 mg/kg IV LD ₅₀ rat >2000 mg/kg (9)
Isoflurane [26675-46-7]	Anesthetic (inhalation)	 Molecular Formula = C ₃ H ₆ ClF ₂ O Formula Weight = 184.49	No UV absorption	Insoluble in water	Oral LD ₅₀ rat >4770 mg/kg IV LD ₅₀ rat >4280 mg/kg (10) Oral LD ₅₀ rat is 4.77 ml/kg (11)
Progesterone [57-83-0]	Progestogen	 Molecular Formula = C ₂₁ H ₃₀ O ₃ Formula Weight = 314.46	UV max absorption at 210 nm, (12) Linear range: 4-60 µg/mL	Practically insoluble in water	Oral LD ₅₀ mouse 1050 mg/kg (13) IV LD ₅₀ mouse 79.5 mg/kg (14)
Felodipine [72509-76-3]	Anti-hypertensive, antianginal	 Molecular Formula = C ₁₂ H ₁₂ Cl ₂ NO ₂ Formula Weight = 384.25	UV max absorption at 234 nm, Linear range: 4-24 µg/mL Other UV absorption at 360 nm, Linear range: 8-60 µg/mL	Insoluble in water	Oral rat LD ₅₀ is 1050 mg/kg IV rat LD ₅₀ is 5.4 mg/kg (15)
Ciclesonide [126544-47-6]	Anti-asthmatic; glucocorticoid	 Molecular Formula = C ₂₉ H ₄₂ O ₆ Formula Weight = 546.69	UV max absorption at 242 nm, Linear range: 4-24 µg/mL	Practically insoluble in water	Oral LD ₅₀ rat is > 2000 mg/kg
Fluticasone Propionate [80474-14-2]	Anti-allergic; anti-asthmatic; anti-inflammatory	 Molecular Formula = C ₂₉ H ₄₂ O ₈ S ₂ Formula Weight = 592.51	UV max absorption at 246 nm, Linear range: 1-15 µg/mL	Practically insoluble in water	Oral LD ₅₀ rat is >2000 mg/kg (16)
Dexamethasone [50-02-2]	Glucocorticoid; antiemetic; diagnosis aid (Cushing's Syndrome, depression)	 Molecular Formula = C ₂₉ H ₄₂ O ₈ Formula Weight = 392.46	UV max absorption at 241 nm, Linear range: 1-30 µg/mL	Soluble in water, solubility at 25 °C in water, 0.1 mg/mL	Oral LD ₅₀ rat > 3000 mg/kg (17-18)
Quinapril Hydrochloride [85441-61-8]	Anti-hypertensive in treatment of congestive heart failure	 Cl-H	UV max absorption at 222 nm, Linear range: 2-10 µg/mL	Freely soluble in aqueous solvents	Oral LD ₅₀ rat 3541 mg/kg (19) IV LD ₅₀ rat 107 mg/kg (20)
Halobetasol propionate [66852-54-8]	Anti-inflammatory; antipsoriatic	 Molecular Formula = C ₂₉ H ₄₂ O ₈ Formula Weight = 494.96	UV max absorption at 234 nm, LOD: 1 µg/mL	Practically insoluble in water	Oral LD ₅₀ rat > 15 mL/kg (21)

Table I (Cont'd): API description, formula, molecular mass, structure, and solubility. LOD is limit of detection, LD₅₀ is the lethal dose of active that kills 50% of a sample population.

Actives* (chemical abstracts registry number)	General indication*	Structure, formula, and molecular mass*	Spectral properties	Solubility*	Toxicity (LD ₅₀ rat)
Flufenamic acid [530-78-9]	Anti-inflammatory; analgesic	<p>Molecular Formula = C₁₄H₁₀F₃NO₂ Formula Weight = 281.23</p>	UV max absorption at 285 nm, Linear range: 5-25 µg/mL	Practically insoluble in water	IV LD50 rat 98 mg/kg (22) Oral LD50 rat 249 mg/kg (23)
Clobetasol Propionate [25122-46-7]	Glucocorticoid; anti-inflammatory; anti-psoriatic	<p>Molecular Formula = C₂₁H₂₉ClFO₃ Formula Weight = 406.97</p>	UV max absorption at 239 nm, Linear range: 2-40 µg/mL	Insoluble in water	Oral LD50 rat > 3 gm/kg (3000 mg/kg) (24)
Terazosin Hydrochloride [63590-64-7]	Anti-hypertensive in treatment of benign prostate hyperplasia	<p>Molecular Formula = C₁₉H₂₉N₃O₄ Formula Weight = 387.43</p>	Bromophenol blue was used for ion pair complex with the drug in 1:1 ratio. UV max absorption at 425 nm, linear range, was 1-10 µg/mL.	Soluble in water, solubility at 25 °C in water, 33.7 mg/mL	IV LD50 rat 277 mg/kg for males and 293 mg/kg for females (25) Oral LD50 rat is 5500 mg/kg (5500mg/kg), IV LD50 rat is 255 mg/kg (26)
Bupivacaine Hydrochloride [38396-39-3]	Anesthetic local	<p>Molecular Formula = C₁₉H₂₉N₃O Formula Weight = 288.43</p>	The UV shows a maximum at 262 nm with a shoulder at 271 nm. There is also significant end-absorption below 250 nm.	Freely soluble in water	IV LD50 rat 6 mg/kg (27)
Warfarin [81-81-2]	Anti-coagulant	<p>Molecular Formula = C₁₉H₁₆O₄ Formula Weight = 308.33</p>	UV max absorption at 280 nm, LOD: 2.5 µg/mL	Practically insoluble in water	Oral LD50 rat: 1600 µg/kg Oral LD50 mice 60 mg/kg and IV LD50 mice 165 mg/kg (28)

*The Merck Index, 15th Edition, The Royal Society of Chemistry, 2013, pp 149-150, 264, 403, 423, 533, 726, 758, 773, 794, 849, 959, 1441, 1492, 1696 and 1868.

Background

The property, structure, and solubility of 15 generic APIs manufactured in Ireland and around the world are listed in **Table I** (7-28). It is often assumed that the API with the lowest solubility is the most difficult to clean. All 15 APIs were evaluated for the cleaning study. Three APIs, including progesterone, flufenamic acid, and quinapril hydrochloride, were selected for further residual determination using UV and FTIR based on the results from the cleaning evaluation (difficult to clean) and the differences in the chemical structures (i.e., steroid, amino benzoic acid, and dipeptide). Testing was performed in Saint Louis, MO.

Critical parameters and lab studies

The critical cleaning parameters and factors affecting cleaning performance have been well defined in the

literature. These include: time, action, cleaning chemistry, concentration, temperature, water quality, surface, soil load and condition, and environmental factors (29). When designing a cleaning process, it is important to design the laboratory studies to accurately represent both the residue in contact with the surface and the condition of the residue during the process and dirty hold phases (30). However, in some processing steps, such as the addition of dry components, mixing high viscosity slurries, and reconstituting dried products, residues may be observed at and/or above the liquid level that are more difficult to clean than the API or the final mixed formulation (31). The APIs evaluated in this study were: atenolol, gabapentin, isoflurane, progesterone, felodipine, ciclesonide, fluticasone propionate, dexamethasone, quinapril hydrochloride, halobetasol propionate, flufenamic acid, clobetasol propionate,

Table II: Laboratory study testing matrix. w/v is weight/volume.

Critical parameter	Testing condition
Time	<ul style="list-style-type: none"> • Change in wash time • Dirty hold time (16 hours)
Action	• Agitated immersion
Cleaning chemistry	• Use of formulated chemistry to improve cleaning efficiency
Concentration	• Change in cleaning agent concentration
Temperature	• Change in temperature of wash solution as needed
Water quality	• De-ionized water
Surface	• 304 stainless steel coupons of 7.5 x 15 cm with a 2B finish
Soil load and condition	• 10 % w/v slurry baked at 80 °C for 16 hours

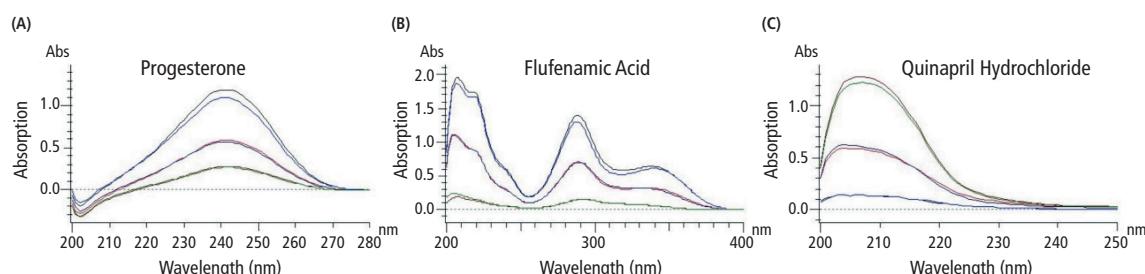
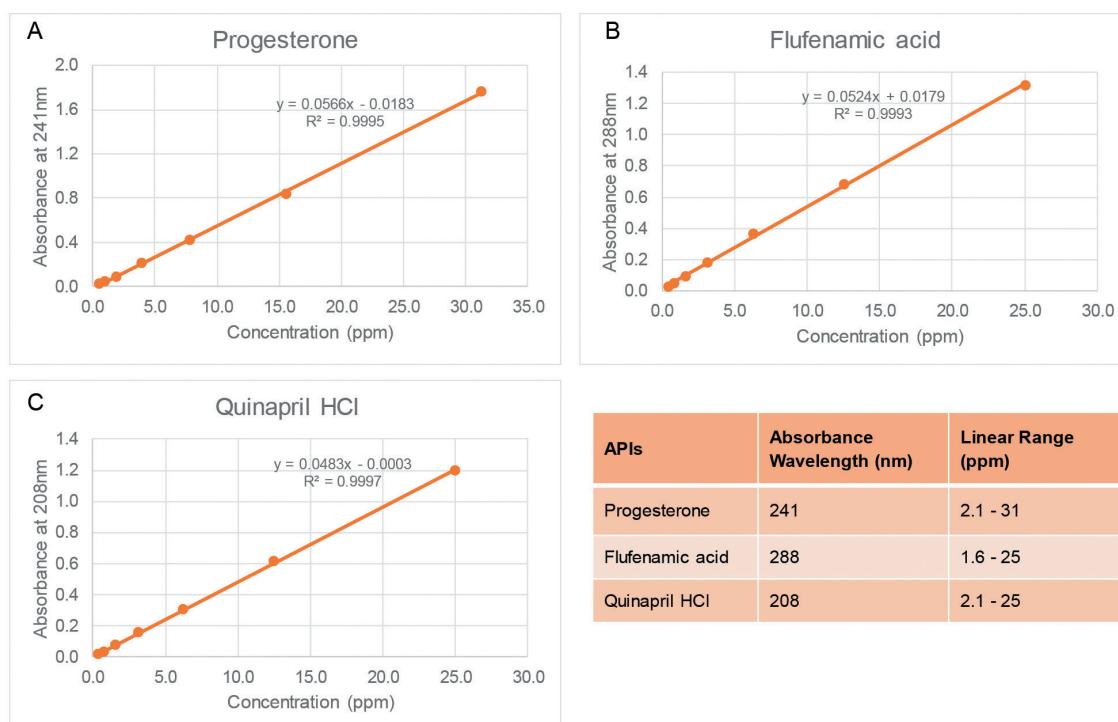
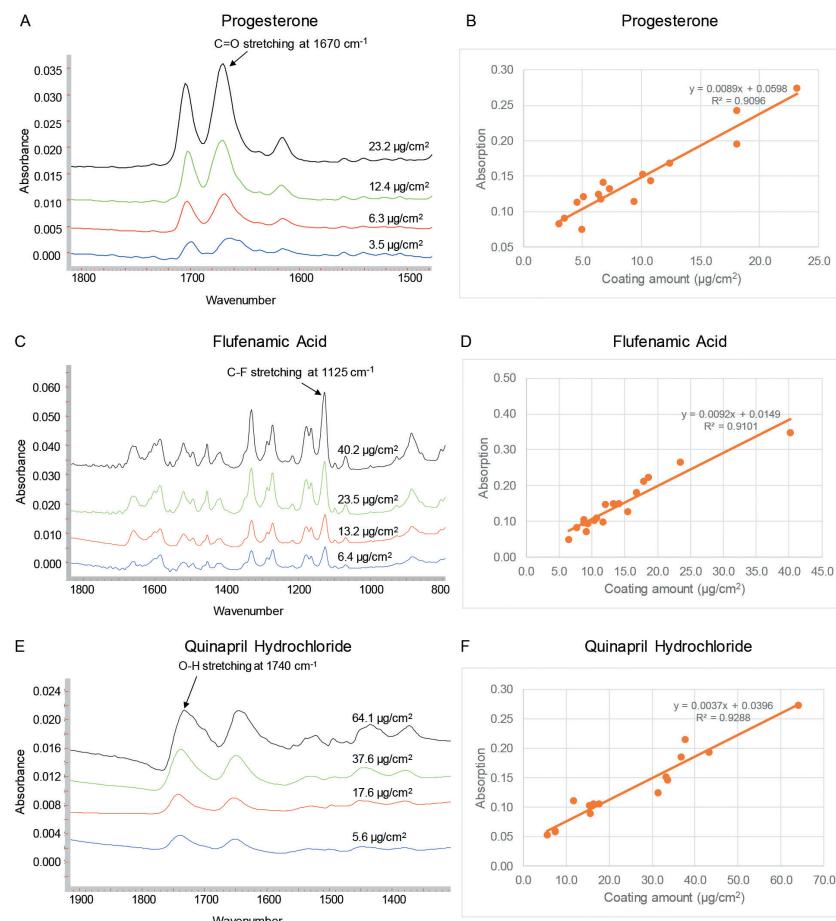
Figure 1: A–C: UV absorption for controls and coupons at different application concentration of progesterone, flufenamic acid, and quinapril hydrochloride. (On each figure, from top to bottom, 10 µg/cm²_ctrl, 10 µg/cm², 5 µg/cm²_ctrl, 5µg/cm², 1 µg/cm²_ctrl, and 1 µg/cm²).**Figure 2: Linearity curves for progesterone, flufenamic acid, and quinapril hydrochloride (HCl).**

Figure 3: Benchtop Fourier-transform infrared spectroscopy (FTIR) spectra and calibration curves of progesterone (A, B), Flufenamic acid (C, D) and quinapril hydrochloride (E, F) on stainless-steel coupons coated with variable amount APIs.



terazosin hydrochloride, bupivacaine hydrochloride, and warfarin, all purchased from Sigma Aldrich. The critical parameters investigated during the cleaning-process design development included varying wash times, cleaning chemistries, cleaning agent concentration, and temperature (**Table II**). The dirty hold time (16 hours), cleaning action (low agitation), water quality (de-ionized), and surface characteristics (304 stainless-steel with a 2B finish) were unchanged for this study.

Analytical testing methods for surface and rinse analysis

To better detect and quantify the API residues present, fast and direct analytical tools with high sensitivity and specificity such as spectroscopy were selected based on the nature of the residues and the level of information required from the analysis (32). Analytical techniques such as UV and FTIR can address these challenges in a time efficient manner.

Ultraviolet spectroscopy (UV) is an easy-to-use high-throughput analytical tool for determining low-level impurities in pharmaceuticals and can be found in nearly

all quality control (QC) labs in manufacturing facilities. Sample preparation is relatively simple and can be used for in-line analysis. UV spectrophotometry helps identify API residues based on absorption maxima. This article will demonstrate the analysis and quantification of selected API residues (**Figures 1A–C and 2A–C**).

FTIR is a nondestructive analytical technique that is helpful for identifying and confirming the structure of an API residue because it provides a complex fingerprint that is specific to a compound. Grazing angle reflectance FTIR is a sensitive method for detection of organic residues on metallic surfaces. It allows an analyte spectrum to be measured directly from a sample surface and can be sampled in the large area; however, the technique has not been well suited for real-time analysis of low levels of surface contaminants. Two different FTIR systems including benchtop and handheld were utilized to demonstrate the analysis and quantification of selected API residues (**Figure 3A–F**).

Laboratory cleaning study procedure (Table III). Testing was performed by agitated immersion at ambient temperature

Table III: Laboratory test. w/v is weight/volume. v/v is volume/volume. RPM is revolutions per minute.

Step	Procedure
1	Dry, clean 304 stainless-steel coupons (7.5 x 15 cm size), with a 2B finish, were weighed on an analytical balance (± 0.1 mg) to obtain the pre-coating weight.
2	Coupons were then coated with a 10% w/v slurry of the sample. The amount of residue per surface area was controlled and recorded.
3	The coated coupon was air-dried at ambient temperature until dry, at least 16 hours.
4	The conditioned coupon was weighed on an analytical balance for a determination of pre-cleaning weight.
5	Each coupon was cleaned by agitated immersion. The 1% v/v cleaning solution (1400 mL) was placed into a 2000-mL glass beaker with a stirrer bar. The beaker was then placed on a digital stir plate set at 180 RPM, which creates a light vortex at the surface. The coated coupon was then placed soil side down into the beaker and the cleaning timer started.
6	The coupon was removed at 15-minute intervals and visually observed for cleanliness.
7	The visually clean coupon was then rinsed, on each side, with tap water for 10 seconds at a flow rate of 2 L/min.
8	Each side of coupon was rinsed with deionized water and examined for a water break-free surface.
9	After water break-free inspection the coupons were dried and then weighed on an analytical balance to determine the post-cleaning weight.

Table IV: UV analysis results for progesterone, flufenamic acid, and quinapril hydrochloride with calculation of percent recovery.

Progesterone	10 $\mu\text{g}/\text{cm}^2$		5 $\mu\text{g}/\text{cm}^2$		1 $\mu\text{g}/\text{cm}^2$	
	Abs241nm	% recovery	Abs241nm	% recovery	Abs241nm	% recovery
Coupon 1	1.144	95.7	0.591	98.3	0.253	94.4
Coupon 2	1.171	98.0	0.576	95.8	0.29	108.2
Coupon 3	1.118	93.6	0.614	102.2	0.244	91.0
Ctrl solution	1.195	-	0.601	-	0.268	-
Flufenamic acid	10 $\mu\text{g}/\text{cm}^2$		5 $\mu\text{g}/\text{cm}^2$		1 $\mu\text{g}/\text{cm}^2$	
	Abs288nm	% recovery	Abs288nm	% recovery	Abs288nm	% recovery
Coupon 1	1.143	82.0	0.69	98.4	0.142	102.2
Coupon 2	1.298	93.1	0.708	101.0	0.143	102.9
Coupon 3	1.174	84.2	0.609	86.9	0.138	99.3
Ctrl solution	1.394	-	0.701	-	0.139	-
Quinapril hydrochloride	10 $\mu\text{g}/\text{cm}^2$		5 $\mu\text{g}/\text{cm}^2$		1 $\mu\text{g}/\text{cm}^2$	
	Abs208nm	% recovery	Abs208nm	% recovery	Abs208nm	% recovery
Coupon 1	1.204	93.4	0.559	94.9	0.115	87.1
Coupon 2	1.256	97.4	0.587	99.7	0.12	90.9
Coupon 3	1.259	97.7	0.601	102.0	0.135	102.3
Ctrl solution	1.289	-	0.589	-	0.132	-

ABS is absorption.

for up to 60 minutes. Coupons were checked in 15 minutes increments. If clean surface was not obtained at ambient temperature, temperature was increased to 45 °C and/or 60 °C.

A coupon was clean if it was visually clean and water break-free, and if its pre-coating weight and post-cleaning weight were equal (0.0 mg residue) (30, 33).

UV rinse solution testing procedure

Preparation of coupons and control solution. API solutions of 100 $\mu\text{g}/\text{g}$, 500 $\mu\text{g}/\text{g}$, and 1000 $\mu\text{g}/\text{g}$ in methanol/water buffer (1/1 weight/weight [w/w] ratio) were prepared. Other choice of solvents such as ethanol can be used. The solvent should

completely solubilize the sample and exhibit only weak UV absorption or UV transparency at the measuring wavelengths. Approximately 0.12 mL (0.1 g) of each solution was applied to each of three stainless coupons (measuring 2.5 cm \times 7.5 cm in size). The solution was applied to a spike area of approximately 10 cm^2 of each coupon, so the applied API level was at 1 $\mu\text{g}/\text{cm}^2$, 5 $\mu\text{g}/\text{cm}^2$, and 10 $\mu\text{g}/\text{cm}^2$, separately (**Table IV**). Blanks were prepared in the same way except buffer was used in place of the API solution. All spiked coupons were dried for approximately 72 hours at ambient conditions on the bench. Control solutions were made by dissolving 0.12 mL of the above solutions into 4.88 mL of methanol.

Table V: Cleaning evaluation using 1% v/v cleaning agent at ambient temperature using agitated immersion cleaning method.

	Condition 1: Neutral detergent	Condition 2: Sodium hydroxide	Condition 3: Sodium hydroxide-based detergent	Condition 4: Potassium hydroxide based detergent	Condition 5: Phosphoric acid detergent
Atenolol	Fail - WBF	Fail - WBF	Fail - WBF	Pass - 15 min	Fail - WBF
Gabapentin	Fail - WBF	Fail - WBF	Pass - 60 min	Pass - 15 min	Pass - 60 min
Isoflurane	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min
Progesterone	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF	Pass - 45 min	Fail - VC/WBF
Felodipine	Pass - 45 min	Pass - 30 min	Pass - 15 min	Pass - 15 min	Pass - 30 min
Ciclesonide	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min
Fluticasone propionate	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF*	Fail - VC/WBF
Dexamethasone crystalline	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF**	Fail - VC/WBF
Quinapril hydrochloride	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF***	Fail - VC/WBF
Halbetasol propionate	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min
Flufenamic acid	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min
Clobetasol propionate	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min
Terazosin hydrochloride	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min	Pass - 15 min
Bupivacaine hydrochloride	Fail - VC/WBF	Fail - VC/WBF	Fail - VC/WBF	Pass - 15 min	Fail - VC/WBF
Warfarin	Pass - 15 min	Pass - 30 min	Pass - 15 min	Pass - 15 min	Pass - 15 min

* cleaned in 15 min at 45 °C

**cleaned in 30 min at 45 °C

***cleaned in 45 min at 45 °C

VC = visually clean, WBF = water break free, min = minutes

Visible residue was consistently observed on the coupon at 1 µg/cm² or above under standard laboratory conditions, which is consistent with published literature (6, 34, 35)

Rinsing coupons and rinse water analysis by UV measurement: each coupon was handled with forceps and hung on a stand above a 50-mL collection beaker. Each coupon was rinsed with 5 mL of methanol repeatedly at room temperature over a two-minute period. The methanol cascaded over the coupon without impingement to the coupon surface (36). Each rinse sample was transferred into 4-dram vials and capped. Samples and controls were analyzed along with blanks for UV measurement. A Hitachi UV-Visible double beam spectrophotometer U-3010 with matched quartz cells (1 cm) was used.

Calculation of rinse recovery is shown in **Equation 1**:

$$(\text{percent recovery, \%}) = \left(\frac{\text{Abs}_{\text{Coupons}}}{\text{Abs}_{\text{Ctrl solution}}} \right) * 100\% \quad [\text{Eq. 1}]$$

Where, AbsCtrl solution is UV absorption of the control solution and AbsCoupons is that of the coupon solution.

FTIR direct surface testing procedure. API solutions of 2 mg/g in 100% ethanol were prepared. The tested APIs were readily soluble in ethanol and the ethanol evaporated quickly at room temperature. The solution was applied to stainless

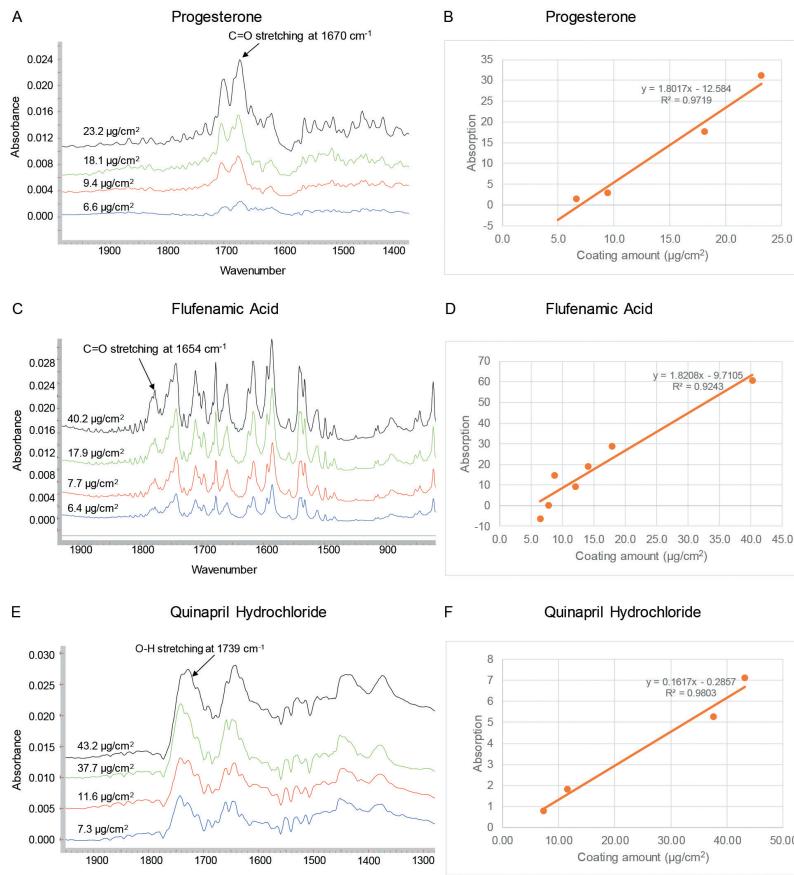
coupons (2.5 cm × 7.5 cm) using a Paasche double-action and internal-mix airbrush, and operated with a small compressor and ballast tank.

It was important that the spray nozzle was not too close to the substrate surface, otherwise excess material can cause solvent to stream off the surface causing a lack of uniformity in the loading. In this study, the size of the multiple-head was 0.64 mm and the distance from the airbrush to the coating surface was approximately 40 cm. As many coatings as necessary were applied. Each coating appeared uniform and dry between each spray. The coating amount was determined by spray times. Blanks were prepared in the same way except ethanol was used in place of the APIs solution. All spiked coupons were dried over the weekend at ambient conditions on the bench.

Grazing angle FTIR spectra were taken at an angle of incidence of 80 ° using an FTIR spectrometer. Spectra were taken after purging in dry air for at least 16 hours and were background-subtracted from a reference sample of freshly cleaned gold, under the same conditions. Spectra were run for 128 scans at a resolution of 2 cm⁻¹, and each coupon was analyzed in triplicate at different locations.

Handheld FTIR spectra were also taken at an angle of 80° using a handheld FTIR spectrometer. The spectra were run for 200 scans at a resolution of 4 cm⁻¹. Three infrared (IR) spectra were collected on each coupon at different location, and the three spectra were baseline corrected and

Figure 4: Handheld Fourier-transform infrared spectroscopy (FTIR) spectra and calibration curves of progesterone (A, B), flufenamic acid (C, D) and quinapril hydrochloride (E, F) on stainless coupons coated with variable amount APIs.



averaged. Because the amount of material deposited by the spray method is difficult to predetermine, an independent method was established to correlate with the true loading of FTIR spectra. After FTIR analysis, each coupon was rinsed with a certain amount of methanol and quantified by UV measurement.

Results

Laboratory cleaning study (**Table V**): a 10% w/v slurry of each of the evaluated residues was baked onto 304 stainless-steel coupons at 80 °C for 16 hours. Different cleaning agents were evaluated to determine the most suitable chemistry to remove the residues. The cleaning agents evaluated included the following:

- 1% w/v formulated neutral cleaner (Condition 1)
- 1% w/v sodium hydroxide (Condition 2)
- 1% v/v formulated cleaner containing sodium hydroxide (Condition 3)

- 1% v/v formulated cleaner containing potassium hydroxide (Condition 4)
- 1% v/v formulated acid cleaner containing phosphoric acid (Condition 5).

Conditions 1, 2, and 5 were not successful in cleaning the residue at 25 °C, 45 °C, and 60 °C for 60 minutes. Coupons were checked in 15-minute increments. Failed coupons had visible residue left. More residues were removed at the higher temperatures; however, that was not enough to completely clean the coupon. Formulated cleaner containing potassium hydroxide was successful in cleaning the API residues using 1% v/v cleaning solution at ambient temperature. Most of the residues were cleaned at ambient temperatures; however, some of the residues required higher temperature of 45 °C and/or 60 °C.

UV rinse solution testing. UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. This technique is simple, rapid,

moderately specific, and applicable to small quantities of compounds.

UV absorption of each control and coupon solution was recorded, and buffer coupon solution was used as blank (**Figure 2A–C**). Progesterone and quinapril hydrochloride exhibit absorption at 241 nm and 208 nm, respectively. Flufenamic acid exhibits three absorptions: a group of strong-light absorption near 210 nm, an absorption at 288 nm, and a weaker band at 340 nm. UV absorption at 241 nm, 288 nm, and 208 nm were used to calculate recovery. On the stainless-steel coupons, the recovery values were found to be over 80% for all three actives (**Table IV**). These results indicate that the UV absorption method could be used as an analytical method to determine residual levels for these pharmaceutical APIs. Note, 14 of the 15 APIs investigated have a reported UV absorption value; refer to **Table I**.

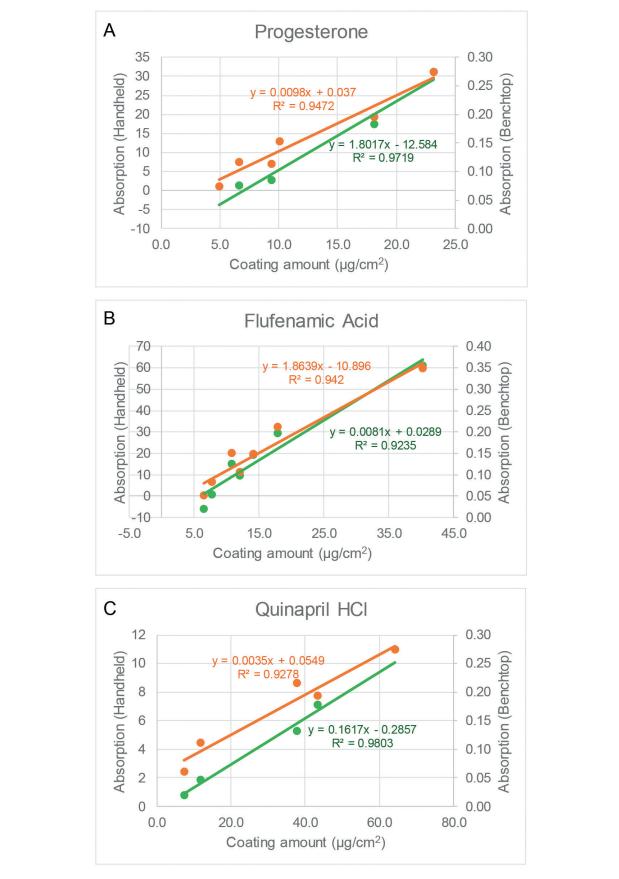
Calibration curves were obtained in a concentration range from 0–200 ppm for all three APIs. Standard stock solutions (200 ppm) were prepared in methanol and were further diluted with methanol to obtain up to 0.4 ppm solutions. The calibration curves were constructed by plotting absorbance versus concentration, and the regression equations were calculated. The response of the APIs was found to be linear in a certain concentration range for each API, and the linear correlation coefficients were all greater than 0.99 for all three APIs (**Figure 2A–C**). The results indicate that the residual level can be estimated directly from the absorption value of the rinsing solution, which could significantly reduce the extent of cleaning validation protocols.

FTIR direct surface testing. The FTIR spectrometer used in this research was coupled by an IR fiber-optic cable to a grazing-angle sampling head with a fixed incidence angle of 80°. The flexible sampling head permits collection of *in situ* spectra from contaminated surfaces.

To achieve a reliable result, the surface loading of the APIs should be as uniform as possible. The IR spectra of the coated coupons were overlaid in **Figure 3**. The intensity of all FTIR peaks increase with the added amount, indicating increase in the adsorption.

The progesterone spectrum has several specific absorption bands caused by different functional groups (**Figure 3A**). The carbonyl (C=O) stretching band at 1675 cm⁻¹ is the strongest absorbance band in the spectrum, which was used to quantify its adsorption. **Figure 3B** shows the results of the adsorption of progesterone at 1675 cm⁻¹ as a function of the coating amount. A good fit between the coating amount and FTIR quantification was obtained. Similar results were obtained for adsorption of flufenamic acid (**Figure 3C** and **3D**) and quinapril hydrochloride (**Figure 3E** and **3F**) on stainless-steel coupons. A handheld FTIR equipped with a grazing-angle sampling accessory was also used to investigate those coupons (**Figure 4**). The carbonyl band was used

Figure 5: Comparison between benchtop and handheld Fourier-transform infrared spectroscopy (FTIR) calibration curves of progesterone (A), flufenamic acid (B), and quinapril hydrochloride (C).



for each model as the quantitative band. Peak intensity was measured for each averaged spectrum. A linear regression was computed for each API (R^2 is >0.90 for all three models). For both FTIR measurements, the absorbance spectra were found to be linearly correlated with the coating amount of APIs (**Figure 5**), which demonstrate the efficacy of using a handheld FTIR spectrometry method in cleaning validation of pharmaceutical manufacturing equipment. This technique delivers accurate, selective surface contamination information in real time to complement current methods and reduce downtime.

Conclusion

Cleaning validation ensures that execution of a standard cleaning procedure consistently delivers a surface which meets pre-established acceptance criteria for cleanliness. A risk-based approach to surface cleanliness incorporates cleanability of the equipment through good engineering design, coverage testing, coupon cleaning trials, surface sampling, visual inspection, and rinse water analysis as

part of the design and qualification stages of the cleaning validation life cycle approach. This article focused on using a direct surface sampling technique (portable FTIR) combined with visual inspection of different APIs to ensure surface cleanliness. The use of direct surface sampling techniques could eliminate swab sampling during routine monitoring or future qualification work with select equipment or tanks. The article also included UV monitoring of the rinse solution for residual APIs. The use of an in-line analytical technique provides an opportunity for continuous monitoring of the final rinse solutions and possibly the wash or post-wash rinses. The incorporation of in-line monitoring of the cleaning process enables future efficiency improvements to the cleaning process as well as real-time continuous monitoring of the cleaning process.

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