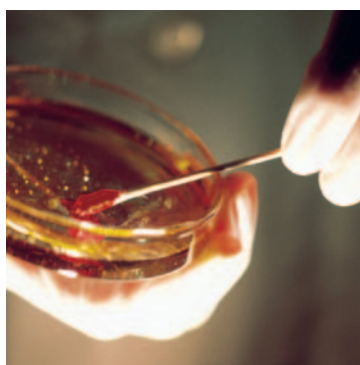# Method Development of
Swab Sampling for Cleaning Validation
of a Residual Active Pharmaceutical Ingredient

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COMSTOCK, INC.

A swab-sampling method was developed for cleaning validation of a residual **active pharmaceutical ingredient** in samples collected after cleaning the sampling suite. A summary of the strategies and results of the method development is presented. The developed extraction method produced an acceptable level of recovery and precision.

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Cross contamination with active ingredients is a real concern. The *Code of Federal Regulations* (*CFR*) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official, or other established requirements" (1). Cleaning validation is required in the pharmaceutical field to avoid potential clinically significant synergistic interactions between pharmacologically active chemicals (2). Since the issuance of the US Food and Drug Administration's "Guide to Inspection of Validation of Cleaning Process" in July 1993 (3), cleaning validations have received increasing attention. Validation is required not only for manufacturing sites, but also for the sampling–filling suite in research and development.

To ensure that the sampling techniques chosen meet the established acceptance criteria, prevalidation feasibility studies and method development must be performed. The two main sampling techniques available for cleaning validation are rinse and swab sampling. FDA prefers swab sampling to rinse sampling (4, 5).

In many pharmaceutical manufacturing sites, a hood is used to sample and aliquot multiple active pharmaceutical ingredients (APIs) on site. After sampling a given lot of API, the sampling or filling suite is cleaned. In the suite where this study was conducted, cleaning is performed by swabbing with water and then 70% isopropyl alcohol (IPA). In many cases, it is also necessary to prove by appropriate analytical testing that the solvent residue is removed from the equipment after cleaning (6). Validation is not required for volatile cleaning agents such as IPA that do not leave a residue (7).

Typically, to verify the effectiveness of API removal, the so-called "worst case" representative API must be determined (8). Therefore, if multiple APIs are used at the manufacturing site, we must demonstrate that the cleaning method effectively removes the API that is the least-soluble in the cleaning solution (in this case, 70% IPA). For this study, all the APIs used at the site were tested, and Compound A was the least soluble in 70% IPA under ambient conditions. Compound A also has chemical features that are typical of a small molecule drug candidate.

A well-conceived recovery study is the keystone of a good

Table I: Results for choice of filter and solvent: swab sampling (n = 3)[a].

Extractable solvent	Filter[b]	Swabbed Sample[c]	Recovery (%)		Filtering Efficiency
			Unfiltered	Filtered	
Mobile phase (TFA–H$_2$O–ACN)	PVDF	10 μg/swab	77.53 ± 5.31	0	0
70% IPA	PVDF	10 μg/swab	17.18 ± 1.17	4.88 ± 1.31	28.04 ± 5.60
50% methanol (pH 6.5)	Nylon	10 μg/swab	84.18 ± 6.02	80.78 ± 4.72	96.01 ± 1.29
Buffer–methanol (50:50) (pH 4.1)[d]	Nylon	2.8 μg/swab	80.38 ± 1.44	80.84 ± 1.26	100.58 ± 0.55
Buffer–methanol (50:50) (pH 4.1)[d]	Nylon	4.0 mg/swab	84.67 ± 2.97	84.91 ± 3.10	100.29 ± .14

[a] Various levels of swabbed sample were investigated. The table only includes typical results.
[b] PVDF and nylon membrane filters (0.45 μm, Pall Gelmann, Pittsburgh, PA).
[c] The swabs used were TX-714A swabs (Texwipe, Pittsburgh, PA).
[d] Prepared by mixing 0.2-M sodium phosphate solution and 0.1-M citric acid solution at a ratio of 30:70 (v/v) (pH 3.5).

Table II: Results for weight of solvent soaked on the tips.

Measurement	Preswabbed/ squeezed (mg)	Preswabbed/ squeezed (mg)	Weight of solvent (mg)
1	9721.80	10,532.07	810.27
2	9836.10	10,705.38	869.28
3	9858.17	10,712.59	854.42
Average			844.66 ± 30.70

Table III: Results for the correlation between volume and weight of the soaked solvent at ambient conditions.

Measurement	Volume (mL)	Weight (mg)
1	0.90	823.07
2	0.90	824.72
3	0.92	846.66
Average	0.91	831.48 ± 13.17

Table IV: Results of the squeezing method

Squeezing Method	Concentration (μg/mL)[a]	Recovery (%)
With fingers[b]	0.746	83.84
Against beaker wall[c]	0.735	82.64

[a] The spiking level used was 7 mg/swab.
[b] The two sides of the swab were squeezed firmly between fingers.
[c] The swab tip was pressed against the beaker wall on all four sides twice.

cleaning validation. The recovery study is carried out to show that the selected sampling procedure is capable to recover the "seeded" drug substance from the surfaces cleaned and that the analytical method, when combined with the sampling method, is capable of identifying the drug substance and is accurate and reliable (4). The design of a suitable sampling procedure and analytical method is challenging. In this study, an HPLC test was developed to quantify Compound A in the sample. The residue acceptable limit (RAL) of Compound A was 10 μg/swab or 1 μg/mL in the swab extract on the basis of a rational estimation (6, 9, 10) and the basic information about the API (11) and sampling hood (12).

The purpose of the study is to summarize the strategies used and experiments performed for the method development of swab sampling to determine a residual API in samples collected after cleaning the equipment.

Results and discussion

Rinse and swab sampling are the two main sampling techniques available for cleaning validation. In addition to being preferred by FDA, swab sampling is a more practical technique for use in the hood at a filling–sampling suite.

Swab sampling is a crucial step in cleaning-sample preparation. The swabbing motion is a physical interaction between the swab and the substrate. The process generally comprises several manual steps, and is an inherently subjective activity that varies from operator to operator. It is essential to have a standardized swabbing motion to ensure that recoveries are replicable regardless of who performs the swabbing.

The operator conducting a swabbing procedure must follow a series of steps:
1. Pretreat the swab(s) in the sample solvent, and squeeze the swab(s).
2. Swab the surface of the tested metal firmly and evenly with one side of the swab(s) in a horizontal direction, and with the other side in a vertical direction back and forth (one stroke back and one stroke forward) to cover the entire area.
3. Cut off the handle of the swab into a centrifuged tube.
4. Use 10mL of sample solvent (also called *recovery solvent* or *extractable solvent*) to extract the drug residue by sonication.
5. Filter the extracted sample and analyze the sample by HPLC.

For validation, it is often impossible to "seed" the drug substance onto the actual equipment because of equipment location, position, or size. Therefore, it is advisable to use coupons (small flat pieces of material) as examples of equipment surfaces for cleaning validation, on the assumption that residue will be evenly distributed over the equipment (7).

The sampling of the coupons must mimic the sampling system used in the actual cleaning process (4). Some of the points to be considered include the number of swabs used, the amount of solvent on each swab, the exact motion of the swab over the surface, the number of strokes over the sampling site, and the amount of time spent swabbing and sonicating.

Various factors involved in the extraction method were investigated. The final sample–solution concentrations established for the method were set to represent ~ 70, 100 and 130 of the RAL solution concentration (*i.e.*, 7, 10, 13 mg/swab). The recov-

ery and its RSD are the parameters used to evaluate the effectiveness and credibility of the cleaning process; the results must confirm the appropriateness of the sampling method. *Recovery* is the percentage of residual material that is actually removed by the sampling technique. Calculating the percentage of recovery is relatively straightforward: one should divide the amount recovered from each surface by the amount seeded, and multiply by 100%. Theoretically, the recovery generated by an ideal method should be close to 100%. Because of the intrinsic solubility of the residue and the nature of the manufacturing surfaces, however, it is generally not possible to achieve recovery beyond a certain level (13). FDA guidelines recommend a minimum of 50% recovery (5).

Choice of filter and solvent

The filter and solvent are critical in cleaning validation. In this study, PVDF and nylon-membrane filters (0.45 μm, Pall Gelman, Pittsburgh, PA) were used. The swabs used were TX-714A swabs (Texwipe, Pittsburgh, PA).

The study showed that using the HPLC mobile phase (TFA/H$_2$O/ACN) as the solvent with PVDF as a filter was a bad choice because of poor filtering efficiency, which is measured by dividing the amount of residue in the filtered sample by the amount of residue in the unfiltered sample and expressed as a percent (Table I). When the solvent used was a cleaning agent (70% IPA), however, recovery was still insatisfactory ($\leq$ 20%) and the filter-
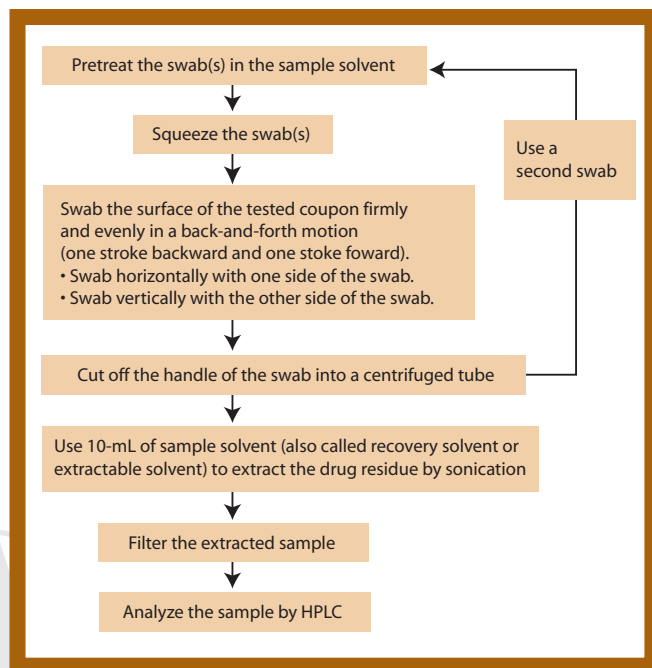
Figure 1: Procedure for sample extraction method.

ing efficiency was still low ($\sim$30%). To improve the recovery and filtering efficiency, a 50% methanol solvent and a nylon filter were tested. The recovery was high ($>$80%), but the filtering efficiency

Table V: Results for batch-to-batch coupon[a].

Coupon batch	Conc. (µg/mL)[b]		Recovery (%)			
	Sample 1	Sample 2	Sample 1	Sample 2	Average	Difference
1	0.796	0.749	89.5	84.19	86.84	3.60
2	0.746	0.736	83.84	82.64	83.24	3.60

[a] 4 × 4 in. 316 stainless steel coupons with a #4 finish, (Globepharma. Inc., New Brunswick, NJ).
[b] The spiking level was 7 mg/swab.

Table VI: Results for 304 stainless steel coupons[a].

Sample	Amount spiked (µg)[b]	Amount recovered (mg)	Recovery (%)	Average recovery (%)	RSD (%) of recovery
1	7.09	6.15	86.63	87.28	1.28
2	7.09	6.08	85.65	87.28	1.28
3	7.09	6.16	86.86	87.28	1.28
4	7.09	6.22	87.64	87.28	1.28
5	7.09	6.25	88.18	87.28	1.28
6	7.09	6.29	88.70	87.28	1.28

[a] 4 × 4 in. 304 stainless steel coupons with a #4 finish, (Alan Steel & Supply Co., CA).
[b] The spiking level was 7 µg/swab.

(~96% per swab sample) still needed improving.

The pH value plays a role in filtering efficiency. The research indicated that the filtering efficiency improved if the pH was changed from 6.5 to 4.1. At the two investigated spiking levels (2.8 and 4.0 mg/mL), there was no significant difference between the values (~100% efficiency) from unfiltered samples and those from filtered samples when using phosphate–citric buffer methanol (50:50) and a nylon filter. The buffer was prepared by mixing 0.2-M sodium phosphate solution and 0.1-M citric acid solution at ratio of 30:70 (v/v) (pH was determined to be 3.5). The apparent pH values of 50% methanol and buffer–methanol (50:50) were 6.5 and 4.1, respectively. Therefore, in the final developed method, the solvent used was phosphate–citric acid buffer–methanol (50:50) and the filter was a nylon filter.

The recoveries for both levels were acceptable ($>80\%$), and the % RSD was very tight ($<4\%$), considering that the established criteria of recovery are $>50\%$, and RSD $<20\%$. As a consequence, it became apparent that the overall developed procedure is considerably more sensitive than required to meet the RAL of 10 µg/swab.

Table VII: The strategies and results of the method development.

Investigated item		Results and conclusions
Filter	PVDF	Nylon was chosen
	Nylon	Nylon was chosen
Solvents	Mobile phase (TFA–H₂O–ACN)	Buffer pH 3.5/methanol
	50% methanol	(50:50) was chosen
	70% IPA	
	Phosphate–citric acid buffer pH 3.5/methanol (50:50)	
Swabbing method	Determination of using one or two swabs	Two swabs used
	Investigation of the solvent volume soaked up by the swab tips	~0.9 mL
Squeezing method	Between fingers or against beaker wall	Squeeze against beaker wall
Effect of the coupon	Batch-to-batch variation	Only ~3.6% variation
	Different lots: stainless steels 304 and 316	Use # 304 (#4 Finishes)

Swabbing method

A literature search and our preliminary study showed that a higher recovery was expected when using two swabs for each coupon rather than one swab (5). An important consideration is the volume of solvent soaked onto the swab tips. The solvent contained on the two tips might increase the actual volume of the extraction solution to more than 10 mL. To check if the volume significantly affected the recovery result, two tips in a container (capped centrifuged tube) were weighed in triplicate before and after swabbing and squeezing. The results showed that the average weight of the solvent absorbed on both tips was ~845 mg (Table II). The volume of solvent absorbed on the tips corresponds to ~0.9 mL, according to the density of the soaked solvent under ambient conditions (Table III).

Because of the variation among operators and individual strokes, the actual volume of the solvent may vary. This is the main reason why the required recovery percentage should be set as a range. It may be concluded, however that the maximum theoretical recovery is not likely to be >92% (10–[10.9 × 100%]).

The use of two swabs may increase the efficiency of absorbing drug residue, but it also results in more solvent contained on the tips. The overall outcome will be a relatively, but not dramatically, higher recovery. The use of more than two swabs however, is not likely to improve recovery, because the contribution of higher efficiency in absorbing the drug residue will not surpass the negative effect of higher solvent volume on the tips. The use of two swabs is ideal.

Squeezing method

It is inconvenient for the analyst to manually squeeze the swab tip after soaking the tips in the sample solvent. In addition, different operators squeeze the swab at various strengths, which leads to variable results. A test was performed to check if any significant difference in recovery exists between manually squeezing the swab between fingers and pressing the swab against the beaker wall. The spiking level used was 7 mg/swab. In the first method, the two sides of the swab were squeezed firmly between the fingers. In the second method, the swab tip was pressed against the beaker wall on all four sides, twice. The samples were tested by HPLC. As shown in Table IV, both methods generated high recovery (>80%), and the recovery levels of the two methods were similar, indicating that no significant difference exists between the two methods.

Effect of the coupon

During the method development, two batches of the metal coupon were tested. The coupons were 4 in. × 4 in. 316 stainless steel with a #4 finish (Globepharma Inc., NJ). The two batches were visibly different. The back of the first-batch coupon was not polished and looked different from the front side, whereas the second-batch

coupon was polished on both sides. In addition, the drops of the spiking solution on the first-batch coupon spread evenly in all directions, while the drops spread out in lines on the second-batch coupon. These apparent batch-to-batch variations could lead to variations in residue recovery.

To test the effect of such variations, the two batches of coupons were used to prepare the swab sample according to the developed extraction method. The spiking level was 7 mg/swab. The results, as shown in Table V, indicated that the recovery on the first-batch coupon was only ~3.6% higher than on the second-batch coupon. Thus, the batch-to-batch variation appeared to be insignificant.

Because the hood in the sampling–filling suite used in the study is made of 304 stainless steel in a #4 finish (12, 14), six coupons of steel were tested. Moreover, to avoid batch-to-batch variation, the 304 stainless steel was purchased in a single order from Alan Steel & Supply Co. (Redwood City, CA). Six coupons of steel were used to prepare the swab sample. The spiking level was 7 mg/swab. The results, shown in Table V, indicated that the coupon generated an acceptable recovery level (>87%) and a low RSD (1.28%).

Conclusions

The study tested the method development of swab sampling for cleaning validation to determine residual API levels in samples collected after cleaning equipment. The strategies and results of the study are summarized in Table VII and the swab-sampling procedure appears in Figure 1. The method was optimized, and the test of recovery and accuracy indicated that the method met the established criteria. Therefore, this method is ready for qualification and subsequent application at a sampling–filling suite.

Acknowledgements

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