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Application of Design of Experiments (DOE) to the Development and Validation of a Swab Sampling Method for Cleaning Validation

Pilar Rodríguez-Loaiza[1], Salvador Namur[2] and Mario González-de la Parra[3*]

[1]School of Chemistry, Universidad Nacional Autónoma de México (UNAM), México City, México
[2]Fundación Liomont, A.C., Mexico City, Mexico
[3]Biokinetics, S.A. de C.V., Mexico City, Mexico; mdelaparra@biokinetics.com.mx

Abstract

Cleaning validation is one of the key elements of the validation program of an active pharmaceutical ingredient (API) manufacturer. One of the most important aspects of cleaning validation is the sampling methods. Swabbing or surface sampling is the subject of this article. The application of sequential experimental designs for the efficient development of a swab sampling method, based on a fractional factorial design followed by full factorial design, is illustrated in this article.

Keywords: Cleaning Validation, Design of Experiments, HPTLC, Swab Sampling

1. Introduction

Cleaning validation is one of the key elements of the validation program of an active pharmaceutical ingredient (API) manufacturer. Cleaning validation in the context of API manufacture may be defined as the process of providing documented evidence that the cleaning methods employed within a facility consistently control potential carryover of product (including intermediates and impurities), cleaning agents and extraneous material into the subsequent product to a level which is below predetermined levels[1].

One of the most important aspects of cleaning validation is the sampling methods. Swab and rinse sampling constitute the two recognized methods for cleaning validation sampling[1,2]. The selection of either of these methods should be consistent with sound scientific judgment and should support the objective of the study, which is to demonstrate that the amount of residual material in the equipment has been reduced to acceptable levels.

Swabbing or surface sampling is the subject of this article and has following main advantages: physical removal of adherent materials such as insoluble residues and the determination of the worst case condition through the sampling sites that may represent worst case locations on the equipment. Because of the nature of this method which employs physical and chemical forces, there are numerous factors that have to be taken into consideration in the evaluation of a swab sampling method[3].

Due to the fact that numerous factors affect this sampling methodology, it was considered convenient to use an approach based on design of experiments (DOE) that has been found to be useful in the determination of the main factors which influence a process and their potential interactions. That is why the DOE has been extensively used in process validation studies[4-6].

Therefore, the objective of this paper was the study the application of DOE for the determination of the main factors and their potential interactions which may influence swab sampling methodology. This is illustrated through the application of DOE to the development and validation of a swab sampling method for water insoluble and difficult to clean drug substance (a corticosteroid) used in topical products. It is important to point out that the identity of the drug substance is not revealed in this article, because of confidentiality and proprietary reasons; and because it is not relevant to the article objectives,

* *Author for correspondence*

since the application of DOE for the development and validation of cleaning sampling methods, should be general in nature.

High performance thin-layer chromatography (HPTLC) was selected as the analytical methodology to determine the residues of the drug substance because it meets the following criteria:

Sensitivity: the ability of the analytical method to detect the drug substance at levels consistent with its acceptance criteria.

Selectivity: the ability of the analytical method to determine the drug substance in the presence of other materials that may also be present in the sample.

Efficiency and cost: the ability of the method of processing many samples per unit of time and at the lowest cost[7].

2. Materials and Methods

2.1 Instrumentation and Reagents

The HPTLC system employed consisted of HPTLC silica gel 60 F_{254} plates (Merck), horizontal elution chambers (10 x 10 cm and 20 x 10 cm, Camag), a semiautomatic applicator Linomat IV (Camag), a densitometer with variable wavelength UV-Visible Scanner 3 (Camag). The chromatographic information was processed by a software WinCats (Camag) version 1.0.

Reagents used were water (Milli-Q purification system, Millipore) and solvents were of the chromatographic grade. The acetone used as a swab extraction solvent was of chromatographic grade and the solubility of the drug substance was found to be 40 mg/mL in this solvent.

2.2 Maximum Allowable Residue (MAR)

The acceptance criterion of the drug substance was determined based on the calculation of the MAR[8]. The MAR is the calculated maximum amount of a residual product (or other contaminant) that is allowed to be carried out into the processing of the first batch of another product. Some organizations use the "worst-case scenario" and estimate the *MAR* as follows:

$$MAR = (STD / SF) \times (SB / LDD),$$

where *STD* = smallest therapeutic dose; *SF* = safety factor; *SB* = smallest batch size of any product made in the same equipment and *LDD* = largest daily dose of any product made in the same equipment.

This equation provides the total residue, usually reported in mg, allowed for all manufacturing and packaging equipment. The MAR, for the present study, was calculated to be 142.86 mg, where: *STD* = 0.1 mg; *SF* = 100 (safety factor for topical products), SB = 1,000,000 mg and LDD = 7 mg.

However, a more useful way of expressing the *MAR*, from the analytical chemistry standpoint is the amount per swab, calculated in the following way:

$$Amount\ per\ swab = (MAR / TSA) \times SA$$

where *TSA* = total surface area and *SA* = sampled area per swab.

In the present case, the amount per swab was calculated to be 62.3 µg. This amount can also be expressed as a concentration by dividing the amount per swab by the volume of solvent using for the extraction of the residue from the swab. In this case, the concentration was found to be 31.15 µg/mL, where the extraction volume was 2 mL.

2.3 HPTLC Method

The analytical and validation parameters of the HPTLC method are given in Table 1.

Table 1. Chromatographic conditions and validation of the HPTLC method

Plate (width)	**100 mm or 200 mm**
Mobile Phase	$CHCl_3$: Ethyl acetate (7:3)
Chamber	Horizontal Elution Chamber 10 x 10 cm or 20 x 10 cm
Wave length	254 nm
Applicator	Semi-automatic applicator Linomat IV (Camag)
Application volume	15 µL
Method Linearity: Correlation coefficient (r) = 0.998 Slope (m) = 0.958 Intercept (b) = 0.505	Method Accuracy: Mean = 100.31% Standard Deviation (SD) = 1.09% Relative Standard Deviation (RSD) = 1.09
Intermediate Precision: Mean = 103.65% Standard Deviation (SD) = 1.65% Relative Standard Deviation (RSD) = 1.59	Limit of Detection (LOD) = 1.26 µg/mL Limit of Quantitation (LOQ) = 2.20 µg/mL

2.4 Recovery Efficiency

Recovery efficiency is the fraction of material originally present on the test surface that is subsequently quantified by the analysis.

Surfaces of either Pyrex® glass or 316 stainless were defined by using Teflon®'s templates to provide the surface areas to be swabbed (16 cm^2 or 25 cm^2). These surface areas were spiked with 1 mL of a standard solution of the drug substance in acetone, at either a low concentration (50 µg/mL) or at a high concentration (150 µg/mL). The solvent was let to evaporate from the surfaces. The surface was then swabbed with either Texwipe® polyester or polyurethane foam heads by either swabbing in a zigzag or in a parallel pattern. After swabbing, each swab was extracted with 2 mL of acetone in a test tube. 15 µL were used for the determination of the recovered residue by the HPTLC method.

The recovery efficiency (recovery %) was calculated as follows:

Recovery % = amount recovered x 100 / amount spiked onto surface

2.5 Statistical Analysis

The data obtained from the experimental designs were analyzed by using the computer package STATISTICA® version 6.1[9]. The level of statistical significance was set at 5% ($\alpha = 0.05$).

3. Results

3.1 Fractional Factorial Design

In order to develop the swab sampling method in an efficient manner, a two sequential step strategy of experimental designs was followed. The first step consisted of a fractional factorial design (screening design) where the interest was on detecting the factors with significant influence on the cleaning sampling method from the numerous factors with potential influence.

Seven factors and their corresponding two levels were considered from both theoretical and practical considerations. These factors, their levels and their justification for inclusion in the experimental design are shown in Table 2.

It is important to mention that a full factorial design with seven factors requires $2^7 = 128$ experimental runs; obviously, this amount of experimentation is excessive for a screening step. Therefore, a fractional factorial design was considered more convenient; in particular, the main effects of seven factors at two levels can be efficiently studied with a $1/16^{th}$ fraction of the full factorial design, which is denoted as 2^{7-4}. This is a minimal design in which the effect of seven factors can be studied with only eight experimental runs, resulting in an important savings of limited resources[10].

This experimental design was performed by randomizing the order of the experiment to protect their

Table 2. Factors included in the fractional factorial design

Factor	Description/Justification	Levels*	
		Low level	High level
Surface	The type of surface. Because the drug substance might be adsorbed differently by different type of surfaces.	Glass	Stainless steel
Soaking	Mechanism of soaking the swab. Because it was suggested that by soaking the head swab with an accurate amount of solvent (by syringe), the recovery would be more reproducible than by immersing the swab in the solvent.	Syringe	Immersion
Residue	The amount of residue adsorbed on the surface. Because a high amount of residue might be difficult to be recovered.	50 µg	150 µg
Pattern	Pattern of swabbing. Because the pattern of swabbing might affect the amount of residue recovered and the recovery consistency between different analysts (e.g., some patterns could be more difficult to perform by a given analyst).	Zigzag	Parallel
Analyst	Differences among analysts. Because analysts have different technical abilities to perform a given task.	A	B
Swab	The type of materials which constitute the swab head. Because the type of materials might affect both the absorption and delivery of the residue by the swab.	Poly	Foam/poly
Area	The area to be swabbed. Because different areas might present some difficulties to be swabbed, thus affecting the recovery of the residue.	16 cm^2	25 cm^2

*The factor levels were coded low (-1) and high (+1). The decision of assigning low or high level to a categorical factor was arbitrary.

Table 3. Fractional factorial design

Swab	Pattern	Soaking	Surface	Area (cm^2)	Analyst	Residue (µg)	Recovery (%)
Poly	Zigzag	Syringe	Steel	25	B	50	19.42
Poly	Zigzag	Immersion	Steel	16	A	150	35.85
Poly	Parallel	Syringe	Glass	25	A	150	68.59
Poly	Parallel	Immersion	Glass	16	B	50	90.18
Foam/Poly	Zigzag	Syringe	Glass	16	B	150	12.33
Foam/Poly	Zigzag	Immersion	Glass	25	A	50	83.93
Foam/Poly	Parallel	Syringe	Steel	16	A	50	38.92
Foam/Poly	Parallel	Immersion	Steel	25	B	150	33.41

results against the effect of time-related variables, such as the increase of technical abilities of the chemists during the execution of the study, and their results are shown in Table 3.

It is important to point out that for a 2^{7-4} design; the eight associated degrees of freedom are spent for the estimation of the grand mean and the effect of the seven variables. Therefore, there are no remaining degrees of freedom for the estimation of the experimental error, thus the usual procedures for determining the statistical significance of the factors, such as analysis of variance (ANOVA) cannot be used. In the absence of the more formal procedures, a Pareto chart of effects (Figure 1) was used for selecting the factors to be further investigated in the next step of experimentation.

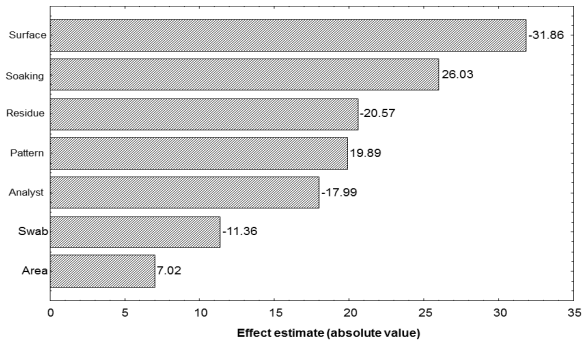

Figure 1. Pareto chart of effects for Recovery (%). Fractional factorial design (2^{7-4}).

3.2 Full Factorial Design

The second step of experimentation consisted of a full factorial design, where all the factor combinations were tested. In this type of design, it is possible to detect both main factor effects and factor interactions.

From the screening design, the three factors with the higher effects were selected: type of surface, the amount of residue and pattern of swabbing. It is important to mention that, although the soaking of the swab showed a high effect in the screening design, it was excluded from further investigation, since the analysts reported that the soaking by syringe was not practical for a routine method.

With these factors, a 2^3 factorial design was selected, resulting in 8 experimental runs. This design was carried out in randomized order and its results are presented in Table 4.

Table 4. Full factorial design

Surface	Residue (µg)	Pattern	Recovery %
Steel	50	Zigzag	32.02
Glass	150	Parallel	91.08
Steel	50	Parallel	88.54
Glass	50	Zigzag	96.10
Steel	150	Zigzag	31.25
Glass	50	Parallel	94.44
Steel	150	Parallel	80.05
Glass	150	Zigzag	93.60

The statistical analysis (ANOVA) is shown in Table 5.

Table 5. ANOVA of Full Factorial Design

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-ratio	p-Value
Surface (S)	2569.011	1	2569.011	436.725	0.030*
Residue (R)	28.577	1	28.577	4.858	0.271
Pattern (P)	1278.662	1	1278.662	217.369	0.043*
S x R	1.445	1	1.445	0.246	0.707
S x P	1498.781	1	1498.781	254.789	0.040*
R x P	9.202	1	9.202	1.564	0.429
Error	5.882	1	5.882		

*Significant ($\alpha = 0.05$)

This analysis indicated that the main effects of the type of surface and the pattern of swabbing, as well as their interaction resulted significant.

4. Discussion

The interpretation of the results of the full factorial design was based on the interaction between the type of surface and the pattern of swabbing. This interpretation is facilitated by looking at the interaction graph (Figure 2), where it is clear that the major difficulties in obtaining high values of recovery were related to the stainless steel surface when it was swabbed by using a zigzag pattern.

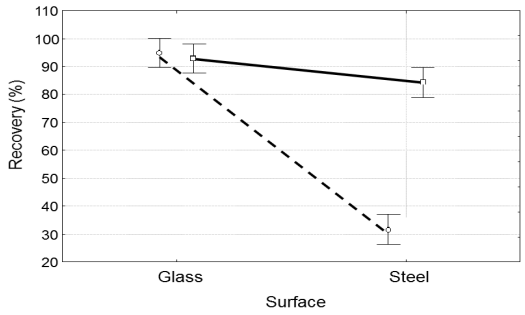

Figure 2. Interaction Graph. Type of surface by pattern of application. Vertical bars denote 95% confidence intervals. Parallel pattern (solid line) and Zigzag pattern (dashed line).

This interpretation suggested that an easier and more robust sampling method could be developed by selecting the parallel swabbing instead of the zigzag swabbing which might cause more difficulties, especially for swabbing stainless steel equipment.

According to the results from both experimental designs, the remaining method conditions were:

Soaking of the swab: immersion because of the reasons mentioned above.

Type of swab: polyurethane foam head because of its availability in large amounts in the company.

Residue amount: the amount of 62.3 µg was selected, because it corresponds to the *MAR* of the drug substance.

Area: the area of 100 cm^2 was selected. It is important to point out, that although this area value is beyond the tested range of 16 cm^2-25cm^2, it was chosen based on satisfactory results from separate studies and because templates for this area value were already available in the company.

When this method was implemented the recovery from both stainless steel and glass surfaces, using 12 replicates per each type of surface were, mean ± standard deviation. For stainless steel 102.9% ± 3.0% and for glass 100.3% ± 4.2%.

In general terms, the recovery was about 100% which means that the recovery factor could be considered as 1 (i.e. calculations will not require any corrections due to the recovery factor). These results represented a great improvement from the initial results obtained during the experimentation stages, showing the benefits of applying the sequential approach of experimental designs, which allows the accumulation of knowledge and experience during each stage of experimentation, which in turns was a key for a successful method validation and implementation.

5. Conclusion

The development of sampling methods for cleaning validation can be affected by numerous factors. A strategy based on DOE is proposed for their development since it is not only more efficient than a strategy based on the traditional study of one-factor-at-a-time. In addition, DOE can reveal the presence of factor interactions that could be very important for the success of the development and validation of the method.

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