D-OPTIMAL APPLICATION AS EFFICIENT TOOL DURING FORMULATION FEASIBILITY STUDIES OF NOVEL MDIs DRUG PRODUCTS

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Summary: The chemical stability of pressurized Metered Dose Inhaler (pMDI) solution formulations can be affected by many variables, such as packaging components, formulation composition, and manufacturing process. Screening all the variables that could potentially have an impact on the chemical stability of the active ingredients can imply a heavy workload, high costs, and a very time consuming step in formulation feasibility. In this paper, a traditional approach for the screening of variables is compared against a multivariate approach. These 4 variables, valve type, canister type, headspace air presence, and storage conditions, were investigated for their influence on active ingredient chemical stability. In particular, 4 different types of canister, 3 different types of valve, the presence or removal of the air in the canister headspace, and 2 different types of storage conditions were considered. All samples were manufactured using two-stage pressure filling equipment; using a validated HPLC/UV method, the active ingredient % residue assay versus the time zero value was evaluated as a main response. One Variable at A Time (OVAT) and Design of Experiment (DoE) approaches were compared. Among the possible DoE, D-Optimal design was selected. To evaluate the chemical stability using the OVAT approach, all 48 (4 x 3 x 2 x 2) possible variable configurations were tested in triplicate (144 tested samples), whereas the application of only the D-Optimal Design would have required merely 48 samples.
It was demonstrated that the application of the D-Optimal Design allows one to obtain easily interpretable information reducing the workload by about 70%.

Introduction: pMDIs are among the most popular devices used in the inhalation field. pMDIs can be formulated as solutions or suspensions depending on the solubility of the active ingredients in the system. In the solution formulations, the active ingredients are dissolved in a suitable co-solvent/propellant mixture and packaged in a canister fitted with a metering valve. The chemical stability of the active ingredients in the solution system is one of the most challenging issues for formulators [1]. Commonly, the first step of formulation feasibility is the screening of several variables which could potentially affect the chemical stability of the active ingredients. The greater the number of variables, the greater the experimental effort, with an increase of time and resource consuming activities. With the traditional OVAT approach, all possible variable combinations are tested. On the contrary, with the DoE approach a limited number of experiments are required to achieve a greater amount of information, since the interactions among variables are also taken into account. The active ingredient contained into the pMDI tested in this study could degrade up to 90% of the initial value or to remain perfectly stable depending on different variables. In this work, the influence on the active ingredients chemical stability monitored as % residue assay versus the time zero value (assay expresses as mg of active ingredient per canister) for the following variables was investigated: type of canister (Can 1, Can 2, Can 3, Can 4), type of valve (Valve 1, Valve 2, Valve 3), headspace air presence (Crimp1 = air present in canister headspace; Crimp2 = air removed from canister headspace), and storage conditions (STC 1 and STC 2), plus the interaction between the latter two variables.
The aim of the study is to demonstrate that, compared to the OVAT approach, the selected D-Optimal Design provides greater and more easily interpretable information, and a significant reduction in workload.

Methods: The tested pMDI samples were prepared by means of two-stage pressure filling equipment (Pamasol Willi Mader AG, Switzerland). The pMDI tested solution is a sponsor formulation. The removal of the air from the canister headspace was obtained by applying vacuum crimping during manufacturing. The chemical stability of the active ingredient was monitored as % residue assay versus the time zero value using a validated RP-HPLC/UV stability-indicating gradient method (HPLC/UV 2690/2695 Alliance, Waters Corporation, Milford, MA, USA, equipped with chromatographic column Kinetex C18, 2.6µm, 100x4.6 Phenomenex, Torrance, CA, USA). For the DoE approach, the results were elaborated using programs written by one of the authors for the Matlab environment (MathWorks, Natick, MA, USA).
OVAT Approach: the OVAT approach investigates the results of each experiment “locally”, without taking into account the response on the whole explored domain. With the OVAT approach, all 48 variable configurations were analyzed in triplicate to estimate the standard deviation of each single configuration. The matrix with all the experimental configurations is reported in Figure 1.
DoE Approach: the DoE approach [2], [3], [4], [5] takes the results of all the experiments executed into account at the same time, with the goal of creating a valid model for the whole experimental domain. This allows for predicting the outcome of the experiments that have not actually been performed.
I In this study, type of valve and type of canister are qualitative variables respectively at four and three levels while headspace air presence and storage conditions are variables at two levels. Taking into account such levels, it is possible to study the variables effect with a linear regression without the estimation of any curvature on the responses (i.e. quadratic effect would be estimable only on quantitative variable with at least three levels). Moreover only the interaction between Crimping and Storage is estimable (the two variables studied at 2 levels).

The model reported in eq. 1 is postulated, from which the model matrix shown in figure 2 is obtained:

$$\%res. = b_o+b_{C1}*X_{C1}+b_{C2}*X_{C2}+b_{C3}*X_{C3}+b_{V1}*X_{V1}+b_{V2}*X_{V2}+b_{Crimp}*X_{Crimp}+b_{STC}*X_{STC}+b_{Crimp*STC}*X_{Crimp*}X_{STC} \quad \text{(eq. 1)}$$

Where:
b_0 = constant
b_{c1}, b_{c2}, b_{c3} = Can variable coefficients
b_{v1}, b_{v2} = Valve variable coefficients
b_{Crimp} = Crimp variable coefficient
b_{STC} = STC variable coefficient
$b_{Crimp*STC}$ = Crimp and STC interaction variable coefficient
In this model, the variables with more than two levels have as many coefficients as the levels minus 1 (3 for Can, 2 for Valve), with the highest coded level as implicit.
Common DoE such as full factorial or fractional factorial do not allow fitting the postulated model. One of the alternative DoE able to estimate each coefficient of the postulated model is the D-optimal design.
D-Optimal Design, a powerful tool to get an experimental matrix with the best possible ratio between experimental effort and information obtained (further mathematical details are not provided because they are out of the scope of this paper).

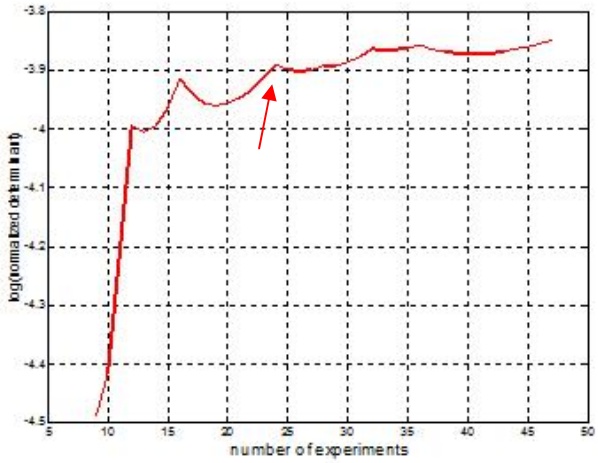
Figure 1: 48 Possible Configurations

Exp	Canister	Valve	Crimping	Storage condition
1	Can 1	Valve 1	Crimp 1	STC 1
2	Can 1	Valve 1	Crimp 2	STC 1
3	Can 1	Valve 2	Crimp 1	STC 1
4	Can 1	Valve 2	Crimp 2	STC 1
5	Can 1	Valve 3	Crimp 1	STC 1
6	Can 1	Valve 3	Crimp 2	STC 1
7	Can 2	Valve 1	Crimp 1	STC 1
8	Can 2	Valve 1	Crimp 2	STC 1
9	Can 2	Valve 2	Crimp 1	STC 1
10	Can 2	Valve 2	Crimp 2	STC 1
11	Can 2	Valve 3	Crimp 1	STC 1
12	Can 2	Valve 3	Crimp 2	STC 1
13	Can 3	Valve 1	Crimp 1	STC 1
14	Can 3	Valve 1	Crimp 2	STC 1
15	Can 3	Valve 2	Crimp 1	STC 1
16	Can 3	Valve 2	Crimp 2	STC 1
17	Can 3	Valve 3	Crimp 1	STC 1
18	Can 3	Valve 3	Crimp 2	STC 1
19	Can 4	Valve 1	Crimp 1	STC 1
20	Can 4	Valve 1	Crimp 2	STC 1
21	Can 4	Valve 2	Crimp 1	STC 1
22	Can 4	Valve 2	Crimp 2	STC 1
23	Can 4	Valve 3	Crimp 1	STC 1
24	Can 4	Valve 3	Crimp 2	STC 1
25	Can 1	Valve 1	Crimp 1	STC 1
26	Can 1	Valve 1	Crimp 2	STC 2
27	Can 1	Valve 2	Crimp 1	STC 2
28	Can 1	Valve 2	Crimp 2	STC 2
29	Can 1	Valve 3	Crimp 1	STC 2
30	Can 1	Valve 3	Crimp 2	STC 2
31	Can 2	Valve 1	Crimp 1	STC 2
32	Can 2	Valve 1	Crimp 2	STC 2
33	Can 2	Valve 2	Crimp 1	STC 2
34	Can 2	Valve 2	Crimp 2	STC 2
35	Can 2	Valve 3	Crimp 1	STC 2
36	Can 2	Valve 3	Crimp 2	STC 2
37	Can 3	Valve 1	Crimp 1	STC 2
38	Can 3	Valve 1	Crimp 2	STC 2
39	Can 3	Valve 2	Crimp 1	STC 2
40	Can 3	Valve 2	Crimp 2	STC 2
41	Can 3	Valve 3	Crimp 1	STC 2
42	Can 3	Valve 3	Crimp 2	STC 2
43	Can 4	Valve 1	Crimp 1	STC 2
44	Can 4	Valve 1	Crimp 2	STC 2
45	Can 4	Valve 2	Crimp 1	STC 2
46	Can 4	Valve 2	Crimp 2	STC 2
47	Can 4	Valve 3	Crimp 1	STC 2
48	Can 4	Valve 3	Crimp 2	STC 2

Figure 2: Model Matrix

Exp	Constant	Can1	Can2	Can3	Valve1	Valve2	Crimp	STC	Crimp*STC
1	1	1	0	0	1	0	-1	-1	1
2	1	1	0	0	1	0	1	-1	-1
3	1	1	0	0	0	1	-1	-1	1
4	1	1	0	0	0	1	1	-1	-1
5	1	1	0	0	0	0	-1	-1	1
6	1	1	0	0	0	0	1	-1	-1
7	1	0	1	0	1	0	-1	-1	1
8	1	0	1	0	1	0	1	-1	-1
9	1	0	1	0	0	1	-1	-1	1
10	1	0	1	0	0	1	1	-1	-1
11	1	0	1	0	0	0	-1	-1	1
12	1	0	1	0	0	0	1	-1	-1
13	1	0	0	1	1	0	-1	-1	1
14	1	0	0	1	1	0	1	-1	-1
15	1	0	0	1	0	1	-1	-1	1
16	1	0	0	1	0	1	1	-1	-1
17	1	0	0	1	0	0	-1	-1	1
18	1	0	0	1	0	0	1	-1	-1
19	1	0	0	0	1	0	-1	-1	1
20	1	0	0	0	1	0	1	-1	-1
21	1	0	0	0	0	1	-1	-1	1
22	1	0	0	0	0	1	1	-1	-1
23	1	0	0	0	0	0	-1	-1	1
24	1	0	0	0	0	0	1	-1	-1
25	1	1	0	0	1	0	-1	1	-1
26	1	1	0	0	1	0	1	1	1
27	1	1	0	0	0	1	-1	1	-1
28	1	1	0	0	0	1	1	1	1
29	1	1	0	0	0	0	-1	1	-1
30	1	1	0	0	0	0	1	1	1
31	1	0	1	0	1	0	-1	1	-1
32	1	0	1	0	1	0	1	1	1
33	1	0	1	0	0	1	-1	1	-1
34	1	0	1	0	0	1	1	1	1
35	1	0	1	0	0	0	-1	1	-1
36	1	0	1	0	0	0	1	1	1
37	1	0	0	1	1	0	-1	1	-1
38	1	0	0	1	1	0	1	1	1
39	1	0	0	1	0	1	-1	1	-1
40	1	0	0	1	0	1	1	1	1
41	1	0	0	1	0	0	-1	1	-1
42	1	0	0	1	0	0	1	1	1
43	1	0	0	0	1	0	-1	1	-1
44	1	0	0	0	1	0	1	1	1
45	1	0	0	0	0	1	-1	1	-1
46	1	0	0	0	0	1	1	1	1
47	1	0	0	0	0	0	-1	1	-1
48	1	0	0	0	0	0	1	1	1

Figure 3: Normalized Determinant vs. Number of Experiments



The normalized determinant as a function of the number of selected experiments is reported in Figure 3 (the normalized determinant can be intuitively interpreted as the amount of information weighted by the experimental effort, and should therefore be maximized). The graph shows that a plateau is reached starting from a subset of 24 experiments (grey rows in Figure 2). Two replicates of each experiment were considered sufficient since the experimental variability is estimated by taking into account the whole data set and not calculated on the replicates of each single experiment as is done with the OVAT approach. The coefficients of the 9-term model reported in Equation 1 were estimated by Multiple Linear Regression Analysis.

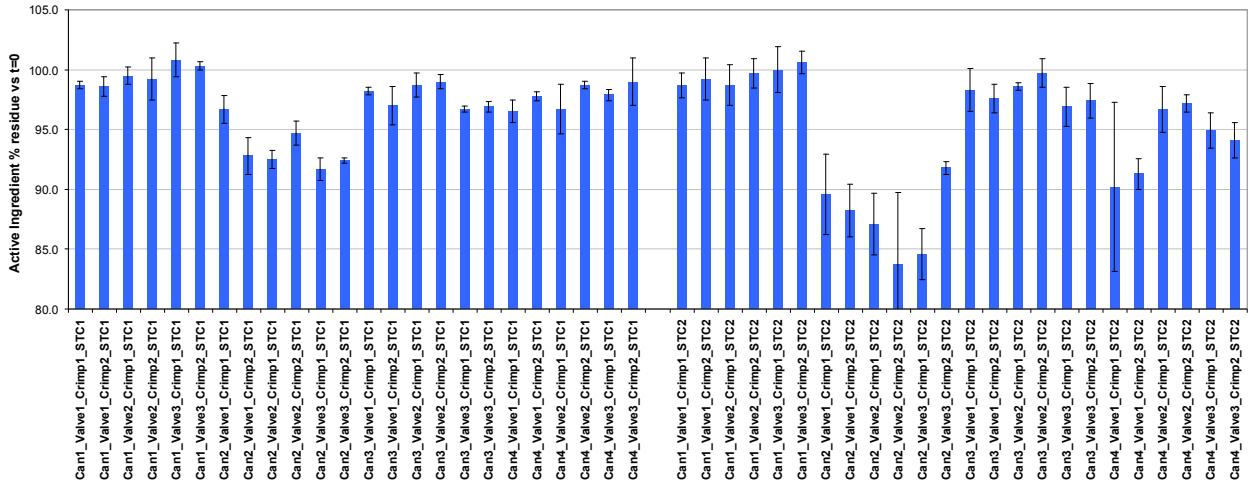
Results:

OVAT approach: The 144 analyzed samples produced the output reported in Figure 4.

The first 24 bars refer to the samples stored under Condition Type 1 (STC1), while the remaining 24 bars refer to Storage Condition Type 2 (STC2). Within the two groups each bar represents a specific combination of the remaining variables "Can Type", "Valve Type", and "Crimping Type".

To understand the influence of the investigated variables, each of the 48 bars should be compared. The only evident outcome from the graph reported in Figure 4 is that Can 2 is the worst canister, while the high number of configurations to be compared could cause difficulties in the interpretation of the results, with the risk of overlooking information related to the significance of the variables; furthermore, it is very difficult to detect and quantify their interactions. Statistical tests for multiple comparisons could be applied, but they could be a weak tool for clearly discriminating among configurations because of the low number of replicates for each experiment.

Figure 4: Active Ingredient % Residue Plot Obtained from 144 samples (OVAT approach)



DoE Approach: Two different models were obtained:

A) All 144 data elements available used (the same data used with OVAT approach, with the substantial difference that all the results are taken into account simultaneously, instead of performing point-to-point comparisons). The obtained output is reported in Figure 5.

B) Using only the 48 data (only a fraction of the ones used for OVAT approach) from the experiments chosen by a D-optimal Design (for each configuration two of the three replicates have been randomly selected). The obtained output is reported in Figure 6.

Figure 5: Active Ingredient % Residue Obtained by analysis of 144 Samples (DoE Approach)

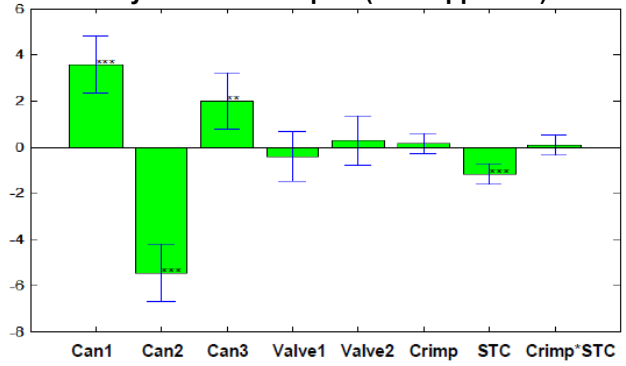
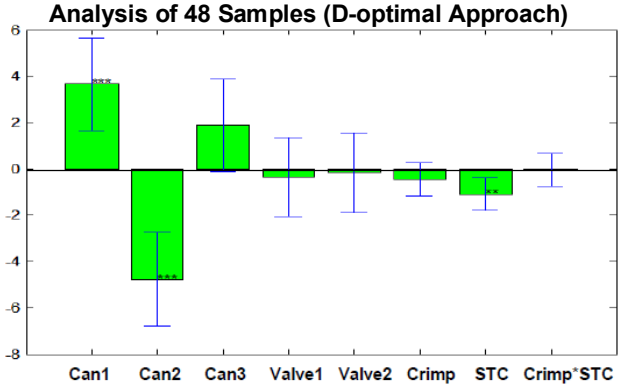


Figure 6: Active Ingredient % Residue Obtained by Analysis of 48 Samples (D-optimal Approach)



The significance of each coefficient is shown by the black stars (*** = p<0.001, ** = p<0.01, * = p<0.05). The blue segments correspond to the 95% confidence interval.

The bars reported for Can 1, Can 2, and Can 3 are the effect of Can 1, Can 2, and Can 3 on the active ingredient % residue compared to Can 4 (implicit variable); they highlight how much the active ingredient % residue is higher (positive values) or lower (negative values) using Can 1, Can 2 or Can 3 instead of Can 4. The bars referring to Valve can be interpreted in the same manner. Bars referring to Crimping and Storage Conditions correspond to the variation of the active ingredient % residue obtained by using Crimp 2 instead of Crimp 1 or STC 2 instead of STC 1. It is possible to see that Can 1 and Can 3 give better results than Can 4 (though there is no significant difference between Can 1 and Can 3), while Can 2 is the worst. The valves and the crimping methods are equivalent, while Storage Condition 2 is evidently worse than Storage Condition 1. Furthermore, it is possible to obtain information about the interaction between the Crimp and STC variables. In this case, the estimated interaction was not statistically significant (see Crimp*STC bar), but it is important to underscore that this evaluation represents additional information which is unobtainable using an OVAT approach.
Comparing Figure 5 (obtained with 144 samples) and Figure 6 (obtained with 48 samples), it is possible to highlight that the quality of information is the same. The main difference is the broader confidence intervals, a direct effect of the smaller number of experiments and therefore of degrees of freedom. This leads to a statistical non-significant effect for Can 3, without changing the final outcome of the experiment**.** Speaking in general terms, the best resulting configuration should be tested and confirmed in order to validate the goodness of the model prediction. In this study, the best configuration found was verified in different studies not here reported.

Conclusion: It has been demonstrated that the D-Optimal design could be a useful tool for pre-formulation screening and formulation feasibility studies. The tool allows one to evaluate sets of well selected experiments to get the most informative combination out of the given variables. The use of D-Optimal design could lead to a strong reduction in the workload (about 70% in the study presented). The proposed approach is particularly useful when there is a high number of possible experiments, when the explored domain is not symmetric, when constraints on the explored domain are present, or when minimizing costs and resources is needed.

References:

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