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# Using Different Experimental Designs in Drug-Excipient Compatibility Studies During the Preformulation Development of a Stable Solid Dosage Formulation

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## Abstract

Different types of factorial experimental designs can be used in compatibility studies of drug development, where many different factors and their interactions should be evaluated to predict their effects on the degradation of the drug substance under study. All possible main and interaction effects of different potential excipients that can constitute the drug product should be evaluated in order to select the best combination of excipients that give the lowest possible degradation, i.e., the most stable drug product. Statistical experimental designs enable the user to obtain the maximum amount of information, i.e., the degradation effects of excipients and their interactions on the stability of the drug substance, on the basis of the smallest possible number of experiments.

The use of full and two different fractional factorial designs is described using a real example where the excipients that stabilize the drug substance or cause as little degradation as possible are selected for a solid dosage formulation. It was shown that the type and the sequence of design used during the studies are also important to get reliable and valuable results. A thorough explanation of the statistical evaluation of data and different presentations of final solutions are given.

**Keywords:** Preformulation studies; Compatibility studies; Stability; Experimental designs; Factor analysis.

## 1. Introduction

The development process of a drug product can be divided into different steps.[1] The first step involves stability studies that enable the product's designer, usually a pharmaceutical technologist, to gain information about the stability of the drug substance and its compatibility with potential additives, called also excipients. The first type of study is called the stress study of the drug substance and the second is the compatibility study.[2–6]

Stress studies of the drug substance are usually carried out at stress storage conditions that involve higher temperature and/or higher relative humidity, the influence of sun or artificial sun light (in "sun test"),[3–6] presence of an oxidation agent such as peroxide or atmospheric oxygen, solutions having different pH values and/or different solvents. The main goal of these stability studies is to obtain information about (1) different factors such as temperature, pH, humidity, light that affect the stability of the drug substance, (2) potential degradation pathways of the substance, (3) main degradation products that appear under degradation of the substance at different stress conditions, and (4) evaluation of stability-indicating nature of the analytical method that is employed to detect different degradation products.

Information about the stability of the drug substance and information about the physical characteristics of the drug substance such as polymorphism or particle distribution help the technologist to design the product, i.e., to define the technological process and to select the excipients that will not or will cause the smallest possible degradation of the drug substance. The influences of excipients on the stability of drug substance are evaluated with compatibility studies.[7] Compatibility studies must be carried out in the early stages of the development, strictly oriented to the final target, i.e., the stable product.

In the past, binary mixtures of drug substance and one excipient were tested at accelerated storage conditions to find out if there was any incompatibility. With binary mixtures no interactions between different possible excipients that can influence the stability of a drug substance are detected. These interactions appear very frequently in solid dosage forms, especially if the drug substance is unstable and its stability depends strongly on different or combined factors, like environmental conditions (water, pH, atmospheric oxygen, light) and composition of the solid dosage form.

Compatibility studies can be more effective by applying more *severe storage conditions* to obtain the results in days instead of in months and by using *statistical experimental designs*[8–12] that prescribe how to vary the values of factors simultaneously in a systematical way in order to obtain maximum information with a minimal number of experiments. Using statistical full factorial experimental designs, all possible effects of factors and their interactions can be evaluated at once.

Experimental designs can save money and time, if the experiments are carried out according to the rules determined by the design and if the results are evaluated properly.

In the above paragraph there are two IF clauses that the performer must be aware of. Firstly, ***all*** of the experiments from an experimental design should be carried out. Otherwise, the factor analysis cannot give reliable results. Therefore, before starting with experiments, the designer has to be sure that all planned experiments can be carried out. Secondly, although numerous different statistical evaluations are available, they all lead to similar conclusions.

In optimization or robustness tests of different analytical methods and technological processes, fractional and Plackett-Burman factorial designs are usually used for the initial quick screening of factors without interactions.[13–18] When the important factors are found, a full factorial design or multi-level fractional factorial design might be set up to find the optimal settings for factors.[19–21] This approach is useful only if the interactions are small, which is usually true for analytical methods and technological processes. However, in compatibility studies of mixtures, interactions are commonly important and significant. If they are not taken into account at the very beginning of the study, the obtained results can lead to a faulty decision. To avoid this, the opposite approach is suggested and explained in this article: to start with full factorial design and a small number of factors between which interactions can be expected and then proceed with fractional factorial designs by replacing the insignificant interactions with new factors.

Since full factorial designs, upon which the number of experiments depends on the number of factors and the number of levels, include a lot of experiments, this can be a considerable disadvantage for a preformulation compatibility study. Fortunately, the relationships between factors and stability parameters are usually linear; therefore, 2-level factorial designs can be applied.[22]

This article is organized as follows; in the next chapter the theoretical items that include the basic definitions concerning experimental designs, the types of experimental designs and factor analysis are given. Subsequently, the theory of statistical experimental designs is illustrated using an example concerning compatibility studies. This example is divided into three steps, each involving one type of factorial experimental design, starting with the simplest and ending with the most complicated one. The calculation of effects, the determination of the experimental error and the ranking of the effects are described in these steps. Finally, some conclusions and suggestions are given.

## 2. Theory

An *experimental system* (system under study) is influenced by numerous different factors such as temperature, humidity, sample composition, etc. The effects of different factors can be determined via responses. The relationship between the experimental system, factors and resulting responses is given in Figure 1.

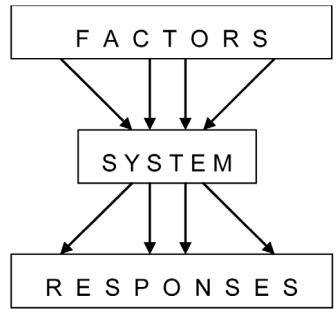


**Figure 1.** Relationship between factors, experimental system and responses.

*Experiments* are actions that are carried out in order to examine the behavior of the system and the influences of factors on the system under study. In statistical experimental design all experiments are determined in advance. This means that in each experiment all the factors have defined values. The main and interaction effects are calculated on the basis of the obtained responses and the factors' signs and the interactions' levels. These calculations are called *factor analysis* and can only be performed if all the experiments of an experimental design are carried out. Therefore, before starting the experiments, one must be sure that all experiments can be carried out; otherwise, the design must be changed by removing some factors or by dividing one experimental design into two or more smaller designs.

The evaluation of calculated effects is possible only if the *experimental error* is determined via variability. The sources of variability are different and can include analytical procedures, technological processes, sample preparation processes, storage conditions, packaging, etc. Only by repeating the analyses can the variability due to the analytical procedure be determined.[23] This is sufficient if only the analytical method is under the examination. This is true for ruggedness, robustness or optimization tests.[24] The best way to determine the overall variability is to use experimental designs where the high-order interaction effects or the main effects of so-called "dummy" factors are the measure of the experimental error.[13,25]

The selection of significant effects from those that are not can be made using different statistical approaches for the evaluation of experimental error that based on the statistical F-test or t-test.[13,22,36] The first test deals with variances and the second with standard deviations. To make the comparison easier a multiplying factor of 2 is introduced. The effect is statistically significant if it is 2-times larger than the experimental error. The so-called Yates analysis gives identical results.[7,22]

In *preformulation compatibility studies* the experimental system is a drug product. Response parameters describe its quality characteristics. Possible parameters are the physical characteristics of the drug product, its dissolution profiles and its stability parameters. They all depend primarily on the technological process and on the composition of the drug product, while each of them can be influenced by several factors. For example, the factors of the technological process are the sequence of adding excipients, temperature, concentration, time of mixing, the velocity of stirring, etc. The factors governing the composition of drug products are the types and contents of all possible excipients that can be found in the final drug product.

The *development of a drug product* can be regarded as a complicated multi-factor multi-level problem that can be solved in iterations. The iteration includes preliminary activities, technical activities and interpretations of results. Before starting any experiment, four preliminary activities must be carried out: (1) identification of all factors that may affect the system. (2) selection of the most significant factors on the basis of known facts, data from literature, experience, etc. (3) determination of the levels of all selected factors, (4) selection of significant response parameters.

These activities should be carried out by experts from different fields (technology, stability, analytics, etc.) so that all aspects are examined carefully before starting experiments. Although time-consuming, these activities can save money and time, if carefully and thoroughly done for further, so called technical activities that involve (1) selection of suitable experimental design, (2) experimental work and (3) factor analysis.

These technical activities are predefined and do not allow any deviations from the selected design.

The interpretation of results is the final activity in the iteration. Following this, the compatibility study is completed or continued with a new iteration by taking into account the information from previous iterations. The number of iterations needed to produce the product of target quality depends on the complexity of the drug product.

Numerous statistical experimental designs are known. Some well-known types of experimental designs include: full factorial designs for screening of factors and all possible interactions,[8,11,24] fractional factorial designs for screening of factors and some interactions,[8,9,24,26] Plackett-Burman designs for screening of factors without interactions,[27,28] different response surface designs (central composite designs, multifactor, multilevel designs, centered cube designs) for modeling,[21,23,29–36] and mixture designs for the examination and modeling of different mixtures.[8]

Since full experimental designs include all combinations of factors, the optimum solution can be selected on the basis of responses without going into detailed calculations of the effects and statistical evaluations of the results. However, fractional and other designs, in which not all combinations of factors are checked, require more complicated statistical evaluations and data treatment to obtain the solutions. Some examples will be given in the following sections.

In addition, it is usually an advantage to start with the simplest 4-experiment design because it can be easily upgraded with new factors and new experiments. There are two possibilities:

(1) Introduction of a new level of the same factor where two additional experiments will be needed.

(2) Introduction of a new factor where two or four experiments should be added, depending on the interaction effect. If the factor is significant new experiments should be added to obtain the full 3-factor 2-level experimental design. This possibility is used in the second step of the experiment.

For fast screening of the influences of factors and their interactions on the system under study, full or fractional multi-factor two-level experimental designs are used.

Full two-level experimental design includes experiments with all combinations of factors on two levels. All experiments should be carried out. At the end each experiment from an experimental design yields one or more responses that are used for factor analysis.

The main disadvantage of full experimental design is the exponential increase of experiments because of the increase of factors, while the main advantage is that it includes all possible interactions.

The two main disadvantages of fractional factorial designs are: (1) *the overlapping of effects*, i.e., where the interaction is replaced with a new factor the calculated effect is the sum of the interaction and main effect and (2)

*incomplete evaluation of interactions*, i.e., only those interactions that are not replaced with new factors can be evaluated.

None of the different fractional factorial designs avoid the overlapping problem completely. In Plackett-Burman designs that were designed for dealing with systems affected by a lot of factors each main effect is overlapped by three or more two-factor interactions.[25,27]

## 3. Experimental and Discussion

During the stress testing of the drug substance it was found out that the substance is susceptible to oxidative degradation and to a wet and acidic environment. In order to develop a stable solid dosage form, different types of stabilizers, fillers and antioxidants and different amounts of binder, disintegrant and aroma are examined during the compatibility studies, which are divided into three different steps.

### 3. 1. Example – First Step (Evaluation of Two Fillers and Two Stabilizers)

Considering information about the stability of the drug substance, the effects of two fillers and two stabilizers are tested at the very beginning of the preformulation compatibility study, as the stabilizer can minimize the effect of acidic filler on the substance under study. The 2-factor 2-level experimental design is used to evaluate the effects of two factors and their interaction. Factor A is a stabilizer and while factor B is filler. Two different types of both excipients are examined, so both factors are qualitative ones. At low level ("–" level) is the stabilizer of type 1 and at high level ("+" level) is the stabilizer of type 2, while the filler of type 1 is at "–" level and the filler of type 2 is at "+" level.

Four mixtures of excipients and drug substance are prepared according to the 2-factor 2-level experimental design given in Table 1. The mixtures are stirred to simulate the technological process. Since the drug substance is sensitive to humidity, oxidation and an acidic environment, the mixtures are stored at an elevated temperature (60 C), at 80% of relative humidity and in contact with atmospheric oxygen for 10 days. The most significant stability parameters that are tested are ***impurity 1*** that appears in acidic and wet environments, ***impurity 2*** that is known as an oxidative degradation product and ***the sum of all impurities***.

The results after the 10-day test and the results of factor analysis are shown in Table 1. For example, the average response at "+" level of factor A for impurity 2 is 0.21 (the average value of 0.18 and 0.24; exp. 3 and 4). The average response at "–" level of factor A for impurity 2 is 0.46 (the average of 0.50 and 0.41; experiments 1 and 2). The difference between the average responses is negative (–0.25), because the average response at "–" level is larger than the average response at "+" level.

On the basis of the absolute values of effects it can be concluded that the interaction AB is the most significant regarding impurity 1 and the sum of all impurities, while the factor A has the largest effect on the content of impurity 2. The sign of the effect points to the level of the factor that gives the highest results. For example, to obtain the highest amount of all impurities, the stabilizer of type 1 ("–" level) and the filler of type 1 ("–" level) should be used. This combination gives the most unstable product, since the highest amount of impurities means the least stable product. Therefore, considering only the main effects the best combination is stabilizer 2 – filler 2. This conclusion is accepted without taking into account the interaction AB, which has the highest effect on the sum of all impurities. Since the interaction AB is negative (the highest value for the sum of all impurities is at "–" level; Table 1, Experiment 2), the smallest result is obtained if the factors are at the same levels. Therefore, the decision made on the basis of main effects is also acceptable regarding the interaction effect (Table 2, last row).

However, decisions are not always so easily reached. For example, looking at the main effects regarding impurity 1 (Table 1), the combination stabilizer 2 – filler 1 should be selected. Since the interaction effect is 14-times and 7-times larger than the main effects of factors A and

**Table 1.** Experimental matrix of full 2-factor 2-level design with three response parameters and calculated effects.

| No. of exp. | A Stabilizer | B Filler | AB interaction | Response parameters | | |
|---|---|---|---|---|---|---|
| | | | | Impurity 1 | Impurity 2 | Sum of all impurities |
| 1 | – (1) | – (1) | + | 0.21 | 0.50 | 2.79 |
| 2 | – (1) | + (2) | – | 1.47 | 0.41 | 4.03 |
| 3 | + (2) | – (1) | – | 1.24 | 0.18 | 3.72 |
| 4 | + (2) | + (2) | + | 0.29 | 0.24 | 2.19 |
| Effects of : | | | | | | |
| Impurity 1 | – 0.08 | 0.16 | –1.11 | | | |
| Impurity 2 | –0.25 | –0.02 | 0.08 | | | |
| Sum of all impurities | –0.46 | –0.15 | –1.39 | | | |

**Table 2.** Results of factor analyses, i.e., the selected types of the stabilizer and the filler, on the basis of three different response parameters.

| Response parameters | A – Stabilizer | B – Filler |
|---|---|---|
| Impurity 1 | 2 →1 (interaction AB) | 1 |
| Impurity 2 | 2 | – |
| Sum of all impurities | 2 | 2 |

B, respectively, the interaction must be taken into account. This means that both factors must be at the same level. Because the main effect of factor B is 2-times larger than the main effect of factor A, the combination stabilizer 1 – filler 1 is preferable regarding the content of impurity 1 (Table 2, second row).

Regarding the effects on the content of impurity 2, only the main effect of stabilizer is important, because the main effect of factor B is not significant (Table 2, third row); it is at the level of experimental error.

Because of the opposite solutions regarding the different responses listed in Table 1, the final selection can be made on the basis of additional aspects that should be examined. The aspects that should be considered for a particular problem are usually different, for example:

*Financial aspect* (selection of cheaper combination of excipients).

*Regulatory aspect* (selection of the more regulatory acceptable ingredients).

*Registration aspect* (selection of ingredients that are allowed in target markets).

*Patent situation* (selection of ingredients and technological solutions that are not under patent protection if a generic drug product is developed).

*Physical parameters of ingredient* that can effect the technological process and the characteristics of the final product.

*Packaging possibilities* to protect products from moisture, atmospheric oxygen, etc.

For example: if protection from moisture is possible, the content of impurity 1 will not increase and the combination stabilizer 2 / filler 2 can be chosen. On the other hand, if storage under nitrogen is possible, the combination stabilizer 1 / filler 1 is preferable.

## 3. 2. Example – Second Step (Evaluation of Binder Content)

In the first step of the example, the effects of two excipients and their interaction on the stability of the drug substance were evaluated. Depending on different technological processes, the formulation can be made with or without a binder. Therefore, in the second step the effect of the binder content is examined. It was decided (1) to use the results from the previous step, (2) to add a third factor C (content of binder) and (3) to perform only four experiments with binder according to the full 3-factor 2-level design given in Table 3. In full 3-factor 2-level experimental design the mixtures without binder ("–" level; "no" binder) are the experiments with numbers 1, 3, 5, and 7 that correspond to experiments 1, 2, 3, and 4 of full 2-factor 2-level design from the first step. The mixtures of experiments 2, 4, 6, and 8 with binder ("+" level; "yes" binder) are prepared on the same way as the mixtures in the first step of the experiments. The experimental and factor analysis results listed in Table 3 are obtained after 10-day treatment at stress conditions.

The experimental error for impurity 1 is 0.06 (= 2 × 0.03). All main effects on Impurity 1 larger than 0.06 are significant. In this comparison the absolute values of effects are taking into account. In Table 3 these are the main effects of factors A and B, that are –0.20 and 0.15, respectively. Regarding impurity 2 and the sum of all impurities, the only significant effects are –0.26 and –0.56 of stabilizer, respectively. In the case of impurity 2, it is compared to the value of 0.06 (= 2 × 0.03) and in the case of the sum of all impurities it is compared to 0.12 (= 2 × 0.06).

**Table 3.** Experimental matrix of full 3-factor 2-level design with three response parameters and calculated effects. Significant effects are bold typed.

| No. of exp. | A Stabilizer | B Filler | C Binder | AB | AC | BC | ABC | Response parameters Impurity 1 | Impurity 2 | Sum of all impurities |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | – (1) | – (1) | – (no) | + | + | + | – | 0.21 | 0.50 | 2.79 |
| 2 | – (1) | – (1) | + (yes) | + | – | – | + | 0.43 | 0.51 | 2.93 |
| 3 | – (1) | + (2) | – (no) | – | + | – | + | 1.47 | 0.41 | 4.03 |
| 4 | – (1) | + (2) | + (yes) | – | – | + | – | 1.60 | 0.40 | 4.25 |
| 5 | + (2) | – (1) | – (no) | – | – | + | + | 1.24 | 0.18 | 3.72 |
| 6 | + (2) | – (1) | + (yes) | – | + | – | – | 1.14 | 0.21 | 3.54 |
| 7 | + (2) | + (2) | – (no) | + | – | – | – | 0.29 | 0.24 | 2.19 |
| 8 | + (2) | + (2) | + (yes) | + | + | + | + | 0.24 | 0.14 | 2.33 |
| Effects of : | | | | | | | | | | |
| Impurity 1 | **–0.20** | **0.15** | 0.05 | **–1.07** | –0.13 | –0.01 | 0.03 | | | |
| Impurity 2 | **–0.26** | –0.05 | –0.02 | 0.05 | –0.02 | –0.04 | –0.03 | | | |
| Sum of all impurities | **–0.56** | –0.04 | 0.08 | **–1.33** | –0.10 | 0.10 | 0.06 | | | |

The selection of interaction effects can be done using a similar procedure as described for main effects, but taking into account also possible highest variability of interactions, since they include the effects and variability of two or more factors.

Using similar procedure as for main effects only three two-factor interaction effects are significant: the effects of interactions AB and AC regarding impurity 1 and the effect of interaction AB regarding the sum of all impurities.

In two-factor interactions the effects of two factors are combined and their variability can increase. Therefore, the main effects of factors that are a part of interaction should be also taken into account during the determination of significance of interactions. The best way to make such evaluation is the comparison of all interaction effects from one experimental design.

In our example the effect of interaction AB is 8-time higher that the effect of interaction AC and the main effect of factor A is higher that the effect of interaction AC. This means that interaction AC can be regarded as not significant.

Taking into account the significant effects, the most stable mixture consists of:

***Stabilizer of type 2***: The main effects of factor A are all negative, i.e., the factor A on "–" level gives higher response parameters than the same factor on "+" level. Since the lowest value of the response parameter means a more stable mixture, the "+" level (type 2) is chosen.

***Filler of type 2***: Although the significant main effect of factor B is positive and therefore the "–" level should be selected, the "+" level (type 2) is selected because of the strong negative effect of the interaction AB that has a significant effect on the content of impurity 1 and on the content of all impurities.

***The content of binder*** is not important: The binder has no significant effects on the stability of mixtures.

The selection of excipients on the basis of main and interaction effects is illustrated in Table 4.

**Table 4.** Results of factor analysis, i.e., the selected type of stabilizer, type of filler and the content of binder on the basis of three different response parameters.

| Response parameters | A – Stabilizer | B – Filler | C – Binder |
|---|---|---|---|
| Impurity 1 | 2 | 1 → 2 (AB inter.) | – |
| Impurity 2 | 2 | – | – |
| Sum of all impurities | 2 | 2 (AB interaction) | – |
| FINAL SELECTION | 2 | 2 | Not important |

## 3. 3. Example – Third Step (Final Selection of All Excipients)

Taking into account the results of the second step of the example, the effects of three additional excipients on the stability of the drug substance are examined. New excipients are as follows:

***Disintegrant*** as a quantitative factor with minimal ("–" level, sign "min") and maximal content ("+" level, sign "max").

***Antioxidant*** as a qualitative factor because two different antioxidants are tested; antioxidant of type 1 at "–" level and antioxidant of type 2 at "+" level.

***Aroma*** as a quantitative factor with minimal ("–" level, sign "min") and maximal content ("+" level, sign "max").

The experimental matrix of full 3-factor 2-level design is used for these experiments. The interactions AC, BC and ABC are replaced with new factors D, E and F because in the second step it was found out that these interactions are not significant for stability. After replacement, the fractional 6-factor 2-level design is obtained. Mixtures are prepared according to the design given in Table 5. Following stress testing, the results listed in Table 5 were obtained. The calculated effects, determined by taking into accout the experimental errors from the second step of experiments, are included in the same Table.

The ranking of combinations of the stabilizer and filler according to the calculated effects are:

stabilizer 2 – filler 2 (the best combination)

stabilizer 2 – filler 1 (similar to the first, if protected from humidity)

stabilizer 1 – filler 1

stabilizer 1 – filler 2 (the worst combination)

Due to the minor effect of binder on the content of impurity 2, it is slightly better if binder is not included in the formulation.

Final conclusions about the other excipients are as follows:

*The content of disintegrant should be maximal if possible*: It has a small but significant negative effect on the content of impurity 1. The final decision depends on the other characteristics of the product, mainly on the dissolution profile.

*No antioxidant*: Antioxidant has no effect on the oxidation process of the drug substance, since its effect on the impurity 2 is not significant. Its negative effect on the content of impurity 1 can be described by different pH values or different water contents of tested antioxidants. Comparing the values of response parameters obtained in the second and in the third step of our experimentations (Tables 3 and 5), it is evident that the values of response parameters of mixtures with antioxidant (third step) are larger. This means that both tested antioxidants destabilize the drug substance.

*No or minimal content of aroma*: The effects of aroma on different response parameters are different, i.e., negative on the content of impurity 2 and positive on the content of impurity 1 and on the sum of all impurities. Since the positive effects are larger, a minimal content of aroma is preferable. This conclusion is not obvious if some other aspects are more important.

The selection procedure of all excipients on the basis of the main and interaction effects from Table 5 is given in Table 6.

**Table 5.** Experimental matrix of fractional 6-factor 2-level design with three response parameters derived from complete 3-factor 2-level design and calculated effects. Significant effects are bold typed.

| | | | | | | | | Response parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **No. of exp.** | **A Stabilizer** | **B Filler** | **C Binder** | **AB** | **D Disinte-grant** | **E Antioxi-dant** | **F Aroma** | **Impu-rity1** | **Impu-rity 2** | **Sum of all impurities** |
| 1 | – (1) | – (1) | – (no) | + | + (max) | + (2) | – (min) | 1.13 | 0.50 | 4.10 |
| 2 | – (1) | – (1) | + (yes) | + | – (min) | – (1) | + (max) | 1.98 | 0.46 | 4.31 |
| 3 | – (1) | + (2) | – (no) | – | + (max) | – (1) | + (max) | 3.19 | 0.26 | 5.58 |
| 4 | – (1) | + (2) | + (yes) | – | – (min) | + (2) | – (min) | 2.56 | 0.39 | 5.38 |
| 5 | + (2) | – (1) | – (no) | – | – (min) | + (2) | + (max) | 1.27 | 0.21 | 3.38 |
| 6 | + (2) | – (1) | + (yes) | – | + (max) | – (1) | – (min) | 1.05 | 0.39 | 3.07 |
| 7 | + (2) | + (2) | – (no) | + | – (min) | – (1) | – (min) | 0.62 | 0.15 | 2.86 |
| 8 | + (2) | + (2) | + (yes) | + | + (max) | + (2) | + (max) | 0.51 | 0.22 | 2.83 |
| Effects of : | | | | | | | | | | |
| Impurity 1 | **–1.35** | **0.36** | –0.03 | **–0.96** | **–0.14** | **–0.34** | **0.40** | | | |
| Impurity 2 | **–0.16** | **–0.14** | **0.09** | 0.02 | 0.04 | 0.02 | **–0.07** | | | |
| Sum of all impurities | **–1.81** | **0.45** | –0.08 | **–0.83** | –0.09 | –0.03 | **0.17** | | | |

The advantages of the use of experimental designs in compatibility studies are not only in the selection of the best possible combination of excipients, but also in the variety of different solutions offered by the analysis of results. These solutions might become useful if some additional restrictions can be applied. Some examples include:

– instead of using aroma ingredients to cover the unsavory taste of tablets, an additional tablet coating can be used,
– the content of the disintegrant depends on the target dissolution results and has no effect on the stability of the product,
– since the antioxidant has negative effects on the stability of the drug substance, a new technological solution that involves the storage of the drug product under nitrogen or the addition of oxygen hunters into the packaging can be considered,
– a technological process without the use of binder if possible.

## 4. Conclusions

Preformulation compatibility studies are necessary in the development of a stable drug product in order to select and evaluate all possible inactive ingredients and their main and interaction effects on the stability of drug substance. Different statistical experimental designs enable the performer to obtain the maximal amount of information on the basis of a minimal number of systematically performed experiments.

As the interactions of ingredients (drug substance and potential inactive excipients) in solid dosage forms are very strong they must be taken into consideration at the very beginning of the drug development, when the selection of excipients starts with compatibility studies. It was shown how a full 2-factor 2-level experimental design that also includes the interaction of two of the most important factors could be enlarged by an additional factor to a full 3-factor 2-level design. It was shown that only one interaction was important, while the others were negligible and were replaced by the new factors. Therefore, on the basis of these conclusions a factorial 6-factor 2-level design was constructed. It enabled the evaluation of six excipients and all their interactions on the stability of the drug substance. Altogether, by performing 16 experiments included in three different factorial experimental designs the type and/or the content of all six excipients were selected and the optimal formulation composition from the stability point of view was determined.

**Table 6.** Results of factor analysis, i.e., the selected type of stabilizer, filler and content of stabilizer on the basis of three different response parameters.

| **Factors:** / **Responses:** | **A Stabilizer** | **B Filler** | **C Binder** | **D Disintegrant** | **E Antioxidant** | **F Aroma** |
|---|---|---|---|---|---|---|
| Impurity 1 | 2 | 1 → 2* | – | Max | 2 | Min |
| Impurity 2 | 2 | 2 | No | – | – | Max |
| Sum of all impurities | 2 | 1 → 2* | – | – | – | Min |
| FINAL SELECTION | 2 | 2 | No – if possible | Max – if possible | No effects on oxidation | Min – if possible |

* due to the negative AB interaction

The effectiveness of the described compatibility studies is increased by the selection of appropriate stress storage conditions and various responses that enable the study of drug substance stability regarding different environmental conditions on micro and macro levels, i.e., inside or outside the formulation (pH, moisture, temperature, oxidation agent). Therefore, brainstorming in teams of scientists from different fields (pharmaceutical technology, analytics, stability, statistics, and legal resources) before starting the experiments can successfully reduce the cost and amount of time needed for formulation development.

Different types of 2-level factorial designs used in the compatibility studies can be expanded to different central composite or multi-level response surface designs.[37] They can be used for modeling and process optimization during the final determination of the formulation composition[32,38,39] and/or technological process.[40–44]

All information obtained during the compatibility studies can also be very useful in the future when come changes of formulation can occur. For example, if the effect of excipient was determined as insignificant during the compatibility studies a new excipient can be introduced without additional tests on final formulation. The only limitation is that the quality characteristics (quantitative parameters) of new excipient(s) are comparable to the examined ones. This approach can thus significantly reduce experimental costs when introducing changes, which occur regularly in pharmaceutical industry, into the existing formulation.

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## Povzetek

Tekom razvoja nekega zdravila lahko pri študiju kompatibilnosti uporabljamo različne eksperimentalne načrte za določanje vplivov različnih faktorjev in njihovih interakcij na kemijsko razgradnjo opazovane zdravilne učinkovine. Na ta način ocenjujemo vplive različnih pomožnih snovi in njihovih interakcij na stabilnost učinkovine z namenom, da izberemo tiste pomožne snovi, ki najmanj doprinesejo k razgradnji učinkovine, ali pa jo celo stabilizirajo. Tako na osnovi minimalnega števila eksperimentov dobimo maksimalno število koristnih informacij o vplivih pomožnih snovi na učinkovino.

Na enem primeru izbora ustreznih pomožnih snovi za končno zdravilno obliko smo prikazali uporabo enega popolnega in dveh delnih faktorskih načrtov. Pokazali smo, da je za dosego smiselnih rezultatov pomembna tako pravilna izbira faktorskih načrtov, kakor tudi njihovo zaporedje uporabe. Podana je tudi obširna razlaga statistične obdelave podatkov, podatki-rezultati pa so na več načinov predstavljeni tudi grafično.