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A factorial design for compatibility studies in preformulation work

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1. Introduction

The purpose of the factorial design described is to find at an early stage in the development of a drug formulation the best suitable excipients from the point of view of drug stability. For this purpose the active substance is mixed with different excipients in powder form and stored at different temperatures for a given period of time. The drug substance is subsequently analysed for degradation products. It is well known that the chemical stability of a drug substance in a binary drug substance-excipient mixture may differ completely from a multi-component drug substance-excipient mixture.

This is due to interactions between the drug substance and the excipients and also to interactions between the excipients (*e.g.* the "acidity" of a solid formulation can be affected by the excipients used, for example magnesium stearate or stearic acid). The statistical planning of experiments can reveal easly such effects and interactions. *Davies* pioneering-work in experimental design and alalysis [1] has been applied to the pharmaceutical sciences by *Sucker*, who used it for determining the optimal quantitative ratios of the excipients in a drug formulation [2].

The factorial design described in this paper on the other hand is used to determine the occurrence of chemical interactions between the excipients and the drug substance and to determine which of the excipients cause incompatibility within a given mixture. Thus the "stabilization" or "destabilization" effects of the individual excipients within multi-component mixtures should be evident.

2. Factorial design

In the pharmaceutical sciences factorial designs have been used for the following purposes:

2.1 *Quantitative factorial designs; e.g.* for the determination of the optimal *quantitative ratios* of excipients in a known qualitative drug formulation.

2.2 *Qualitative factorial designs; e.g.* for the determination of the optimal *qualities* (different suppliers) of a known quantitative drug formulation.

The factorial design which will be described and which was used for the preformulation compatibility studies is, according to 2.2, a qualitative one which uses, instead of different qualities of the same excipients, chemically different excipients for the same pharmaceutical purpose. As factors for this design, pharmaceutically important *functional* components for solid formulations were chosen: filler, lubricant, disintegrant, binder, etc. These pharmaceutical factors represent the framework of the design for solid formulations. The excipients which fulfil the above-mentioned functions may differ considerably in their chemical properties (*e.g.* as a binder: polyvinylpyrrolidone, gelatine). As a consequence the detection of "effects" and "interactions" in the factorial design is facilitated.

The factorial design developed consists of 5 factors A, B, C, D, E, corresponding to a hypothetical pharmaceutical formulation with four excipients (filler, lubricant, disintegrant, binder). The fifth factor E, represents the humidity in a wet granulation technique. This factor was confounded [1] with the unlikely significant interaction ABCD. According to the definition under point 2.1 factor E is the only quantitative one.

Factorial design

Factor	Level	Concentration [%w/w]
A (filler)	(—) lactose	70
	(+) mannitol	70
B (lubricant)	(—) stearic acid	5
	(+) magnesium stearate	5
C (disintegrant)	(—) maize starch	20
	(+) microcrystalline cellulose (Avicel®)	20
D (binder)	(—) polyvinylpyrrolidone	5
	(+) gelatine	5
E (humidity)	(—) no water added	+ 0
	(+) with water added	+ 3

This design gives 16 different powder mixtures:

No.		A	B	C	D	E
1	e	—	—	—	—	+
2	a	+	—	—	—	—
3	b	—	+	—	—	—
4	abe	+	+	—	—	+
5	c	—	—	+	—	—
6	ace	+	—	+	—	+
7	bce	—	+	+	—	+
8	abc	+	+	+	—	—
9	d	—	—	—	+	—
10	ade	+	—	—	+	+
11	bde	—	+	—	+	+
12	abd	+	+	—	+	—
13	cde	—	—	+	+	+
14	acd	+	—	+	+	—
15	bcd	—	+	+	+	—
16	abcde	+	+	+	+	+

3. *Experimental procedure*

For the straightforward quantification of the degradation products a ^3H labelled drug substance was investigated. The test samples were prepared using the 16 different 4-component powder mixtures as carriers. To 1 g of each mixture 10 mg of the drug substance was added and thoroughly mixed in a Turbula mixer*. This amount of drug substance contained 200 μCi ^3H. As described by the factorial design, 30 μl of deionized water was suspended from the caps of the containers before sealing. A set of 16 samples was stored at 50° for 4 weeks. We assumed that at 50° the water evaporated to produce a uniformly humid atmosphere. A second set of 16 samples, which was to serve as a reference, was stored at 4°. In the case of the reference samples no water was added. After storage the radioactive drug substance was extracted from the mixture and analysed by TLC. For the quantification of the degradation products a modified Berthold Scanner II with a Geiger counter was used (direct evaluation technique) [3]. The experimental method and the automated measuring equipment will be described in more detail in a separate paper.

4. *Results of Yates analysis*

The experimental results of table 1 were analysed using the Yates method [4] (see table 2 and 3).

Table 1

Results of radiochromatographic analysis

Mixture No.	Test samples (stored at 50°) % intact drug substance	Reference samples (stored at 4°) % intact drug substance
1	59.6	100.0
2	86.4	98.3
3	95.0	98.7
4	97.0	96.5
5	83.4	96.6
6	53.8	96.7
7	93.7	98.5
8	99.7	96.9
9	54.1	97.9
10	45.8	99.0
11	92.8	95.3
12	96.1	98.0
13	53.6	98.7
14	64.7	99.6
15	94.0	96.4
16	96.3	97.2
	mean and standard deviation	97.8 ± 1.3

* Turbula Type T 2 A, W. A. Bachofen, Basle, Switzerland.

Table 2

Test samples

Effects, aliases, interactions	Effects, aliases, interactions (X 8) R_i	Comments: The chemical stability of the drug substance is positively influenced by
Total	1266.0	
A	13.6	
B	263.2	magnesium stearate
AB	13.6	
C	12.4	
AC	- 34.0	(at the limit of significance)*
BC	- 6.8	
ABC, DE	40.0	PVP-no water added
D	- 71.2	polyvinylpyrrolidone (PVP)
AD	3.2	
BD	58.8	gelatine-magnesium stearate
ABD, CE	- 8.0	
CD	27.2	
ACD, BE	70.8	magnesium stearate-humidity
BCD, AE	- 27.2	
ABCD, E	- 80.8	no water added

* Not interpreted as A and C are not significant

$$(R_{i\,LIMIT} = 2\sqrt{\frac{16.26}{5}[(-2.9)^2 + (3.7)^2 + (-4.5)^2 + (-6.3)^2 + (-0.5)^2]}$$
$$= \pm 32.7)$$

Table 3

Reference samples

Effects, interactions	Effects, interactions (X 8) R_i
Total	1564.3
A	0.1
B	- 9.3
AB	- 0.7
C	- 3.1
AC	0.3
BC	4.1
ABC	- 2.9
D	- 0.1
AD	10.9
BD	- 7.3
ABD	3.7
CD	6.5
ACD	- 4.5
BCD	- 6.3
ABCD	- 0.5

5. *Interpretation of the Yates analysis and discussion*

To check the significance of the results, the radiochromatographic results of the reference samples stored at 4° (unconfounded design) were also subjected to Yates analysis (table 3). In this case it can be assumed that the higher order interactions (order ≥ 2) represent the error of the method. Thus a given result R_i of Yates analysis (table 2) is significant (confidence level > 99%) if the following expressions are valid (F-test of sum of squares $SQ = \frac{R_i^2}{n}$ [2]):

$$\frac{R_i^2}{n} > \frac{4}{n \cdot m} \sum_{i=1}^{m} (I.A.)^2 \, F(1, m) \qquad (1)$$

$$|R_i| \geq 2\sqrt{\frac{1}{m} \sum_{i=1}^{m} (I.A.)^2 \, F(1,m)} \qquad (2)$$

n = number of experiments (reference samples)
m = number of interactions I.A. (reference samples)
I.A. = Interaction (reference samples)
F (l, m) = F-value (99% confidence level) of the Fisher distribution

Factor 2 on the right side of the expression (2) takes account of the confounding of the fifth factor, *i.e.* to eliminate the possibility that two interactions which are just insignificant, such as ABD and CE, give a significant result, when added together. This test of significance gave the results shown in table 2.

An alternative method of testing significance based on the reproducibility of the radiochromatographic results would yield a much higher number of significant results. This fact is due to the relatively small experimental error in this method, which does not take into account systematic errors originating from non-uniform storage conditions, *i.e.* content uniformity of the drug, relative active surface of the excipients, content of "intrinsic" humidity or organic solvents.

For these reasons the above mentioned rather severe test of significance with a confidence level of > 99% was applied.

Table 2 shows that the application of a factorial design yields important information from the compatibility studies between the drug substance and the excipients in multicomponent mixtures.

Detailed interpretation of the Yates analysis (table 2) gives the following results:

5.1 *Best excipient mixtures*

Mannitol–magnesium stearate–maize starch–polyvinylpyrrolidone
Mannitol–magnesium stearate–Avicel®–polyvinylpyrrolidone
Lactose–magnesium stearate–Avicel®–polyvinylpyrrolidone
Lactose–magnesium stearate–maize starch–polyvinylpyrrolidone

5.2 *Excipients to be recommended*

Lactose, mannitol, maize starch, Avicel®, polyvinylpyrrolidone, magnesium stearate.

5.3 *Important Interactions*

In the presence of humidity magnesium stearate has a stabilizing effect on the drug substance. This may be due to the fact that the drug substance is more stable in an alkaline environment. (No degradation was found after storage of the drug substance in a solution pH = 8 at 50° for 4 weeks).

To improve the compatibility of gelatine it should be combined with magnesium stearate.

5.4 Incompatibilities

The drug substance is incompatible with stearic acid (strong incompatibility) as well as gelatine and sensitive to moisture.

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Summary

The purpose of the factorial design for compatibility studies between drug substance and excipients, as developed by the authors, is to investigate the properties of the excipients in multi-component mixtures.
The factorial design makes possible the detection and quantification of the chemical interactions within multi-component excipient mixtures, as well as indicating the excipients which are responsible for them.
Thus, compared with tests of binary drug-substance excipient mixtures, much more relevant information concerning the stability of drug substance in a multi-component solid formulation becomes available.

Zusammenfassung

Der vorliegende Faktorenversuchsplan wurde entwikkelt, um die Kompatibilität eines Wirkstoffes in einer Pulvermischung aus mehreren Hilfsstoffkomponenten quantitativ in Abhängigkeit von den einzelnen Hilfsstoffen zu bestimmen. Die statistische Versuchsplanung bietet sich hier als Methode der Wahl an, da insbesondere auch die gegenseitige Beeinflussung der Hilfsstoffe (Wechselwirkungen) bezüglich der Kompatibilität des Wirkstoffes im Hilfsstoffgemisch aufgezeigt wird.
Verglichen mit Kompatibilitätsuntersuchungen binärer Wirkstoff-Hilfsstoffmischungen kann dabei wesentlich mehr Information über die Stabilität des Arzneistoffes in einer festen Zubereitung gewonnen werden.

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