https://doi.org/10.31925/farmacia.2021.5.6 **ORIGINAL ARTICLE**

IMPLEMENTATION OF EXPERIMENTAL DESIGN METHODOLOGY IN THE COMPATIBILITY STUDY OF HYDROCORTISONE WITH SELECTED EXCIPIENTS USED IN SOLID DOSAGE FORMS

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Abstract

The aim of the study was to assess the usefulness and the possibility of application of experimental design methodology (DoE) in defining the qualitative composition of a pharmaceutical formulation containing hydrocortisone as the active pharmaceutical ingredient. Eight multi-component mixtures were prepared according to the matrix generated by means of the fractional factorial design. Four types of excipients (binders, disintegrants, fillers and glidants) were used as input variables. In order to accelerate the possible chemical changes in the chemical structure of hydrocortisone, the prepared mixtures were exposed to the temperature of 50°C and humidity of 50% RH for 3 months. The evaluation of the chemical interactions, expressed by the hydrocortisone degradation, was monitored using high-performance liquid chromatography. The DoE approach allowed for the selection of optimal excipients. The fractional factorial design as a knowledge discovery method provides useful clues for the formulation analysts and thus this approach can be taken into consideration in order to support rational decision making in the field of pharmaceutical technology.

Rezumat

Scopul studiului a fost de a evalua utilitatea și posibilitatea aplicării unui plan experimental (DoE) în definirea compoziției calitative a unei formulări farmaceutice care conține hidrocortizon ca ingredient farmaceutic activ. Au fost preparate opt amestecuri multi-componente în conformitate cu matricea generată prin intermediul designului factorial fracțional. Au fost utilizate ca variabile de intrare patru tipuri de excipienți (lianți, dezintegranți, substanțe de umplere și agenți de alunecare). Amestecurile preparate au fost expuse la temperatura de 50°C și umiditate de 50% timp de 3 luni, pentru a accelera apariția posibilelor modificări chimice în structura chimică a hidrocortizonului. Evaluarea interacțiunilor chimice, exprimată prin degradarea hidrocortizonului, a fost monitorizată utilizând cromatografia lichidă de înaltă performanță. Abordarea DoE a permis selectarea excipienților optimi. *Design*-ul factorial fracțional oferă indicii utile în procesul de formulare și, prin urmare, această abordare poate fi luată în considerare de analiști, pentru a sprijini luarea deciziilor raționale în domeniul tehnologiei farmaceutice.

Keywords: experimental design methodology, hydrocortisone, formulation studies

Introduction

The assessment of the chemical interactions of an active pharmaceutical ingredient with excipients is extremely important at the drug product development stage. However, there are no clearly defined guidelines regarding the method and conditions of conducting pharmaceutical raw materials compatibility tests. The research articles describe the approach using binary mixtures consisting of an active pharmaceutical ingredient and a studied excipient in a 1:1 weight ratio or in a non-equilibrium ratio. Increasingly, in compatibility studies of pharmaceutical ingredients, multi-component mixtures are prepared in order to reflect in a higher manner the real composition of the target drug product [3, 10, 14, 19].

The regulatory agencies (United States Food and Drug Administration, European Medicines Agency) recommend the implementation of so-called *Quality by Design* (QbD) concept at the drug product development stage [8]. The development of the pharmaceutical formulations in accordance with the QbD concept allows gaining knowledge about the drug product and better understanding the impact of physicochemical properties of pharmaceutical raw materials on the final drug product quality [6, 12, 16]. The *Quality by Design* concept is a scientific, rational, knowledge-based approach to the drug product development [2, 7, 15, 17, 23].

Regarding the current trends in industrial pharmacy, this paper considers the application of experimental design methodology (Design of Experiments (DoE), Experimental Design (ED)) as a tool enabling the implementation of the *Quality by Design* concept at the formulation studies [1, 4, 9, 13, 21]. As there is no literature data on application of this methodology for

defining the qualitative composition of hydrocortisone formulations, in this work, attention is directed toward DoE as a potentially useful tool for hydrocortisone formulation development. The aim of the research studies was to assess the usefulness and the possibility of application of the fractional factorial design in defining the qualitative composition of a pharmaceutical formulation containing hydrocortisone as the active pharmaceutical ingredient.

Materials and Methods

The hydrocortisone was used as a model active pharmaceutical ingredient. Potato starch, sodium croscarmellose, lactose, microcrystalline cellulose, hydroxypropylmethyl cellulose (hypromellose), hydroxyethylcellulose, magnesium stearate and stearic acid as excipients were used. All excipients were obtained from Rettenmaier Poland. Hydrocortisone was provided by Pharma Cosmetic (Poland).

The whole experimental work was designed using the experimental design methodology (DoE). Eight different multi-component mixtures were prepared in a porcelain mortar according to the matrix generated with the use of the fractional factorial design. Four types of excipients (binders, disintegrants, fillers and lubricants) were used as input variables. Each input variable appeared on two levels of values (binary input variables). The output variable (the degree of hydrocortisone degradation) was a continuous variable, described by a numerical value. Table I shows all combinations of multi-component mixtures for which the hydrocortisone degradation was determined. In order to minimize the risk of systematic errors, a randomization of a plan was applied. The STATISTICA 10.0 software (StatSoft, Tulsa, OK, USA) was used to construct the experimental design as well as to analyse the data.

Table I

Qualitative composition of multicomponent mixtures according to the fractional factorial design 2^3 and the chromatographic results (hydrocortisone degradation)

Fractional factorial design 2^3					
	Filler	Disintegrant	Binder	Lubricant	Hydrocortisone degradation [%]
1	lactose	potato starch	hypromellose	magnesium stearate	22.88
2	microcrystalline cellulose	potato starch	hypromellose	stearic acid	24.47
3	lactose	croscarmellose sodium	hypromellose	stearic acid	16.97
4	microcrystalline cellulose	croscarmellose sodium	hypromellose	magnesium stearate	25.26
5	lactose	potato starch	hydroxyethylcellulose	stearic acid	26.42
6	microcrystalline cellulose	potato starch	hydroxyethylcellulose	magnesium stearate	28.96
7	lactose	croscarmellose sodium	hydroxyethylcellulose	magnesium stearate	26.83
8	microcrystalline cellulose	croscarmellose sodium	hydroxyethylcellulose	stearic acid	25.70

* each mixture included hydrocortisone as the active pharmaceutical ingredient

800 mg of each mixture (containing 250 mg hydrocortisone) was prepared in a porcelain mortar. According to the literature data, disintegrants constituted 5% (w/w), binders 5% (w/w), and lubricants 1% (w/w) of the mixture. The mass of the mixtures was filled up to 800 mg using the appropriate filler (lactose or microcrystalline cellulose).

In order to accelerate possible chemical interactions, the prepared mixtures were exposed to the temperature of 50°C and humidity of 50% RH for a period of 3 months (climate chamber - Binder, KBF P 240 model). The evaluation of the chemical interactions, expressed as the degradation of hydrocortisone in mixtures, was monitored using high performance liquid chromatography (HPLC, Shimadzu). The hydrocortisone content in the mixtures exposed to stress conditions (50°C/50% RH) was determined after 3 months by chromatographic method with the use of a calibration curve ($R^2 = 0.9994$).

HPLC method

A reversed phase HPLC (high performance liquid chromatography) method for the determination of hydrocortisone in prepared mixtures was developed with the aid of a HPLC system (Shimadzu, Japan) equipped with two LC-20AD solvent delivery pumps, a DGU-20A degasser and a diode array detector (SPD-M20A). Chromatographic experiments were performed on a Kinetex EVO C18 column (4.6 mm x 100 mm, 5 μm), filled with silica gel modified with octadecylsilane (C18) groups. The mobile phase consisted of acetonitrile and water in a volume ratio of 60:40. HPLC-grade acetonitrile was purchased from POCh (Poland). Ultrapure water was obtained from a Milli-Q water purification system from Millipore (USA). All chromatographic experiments were performed in the isocratic mode. The flow rate was set to 0.4 mL/min and the injection volume was 5 μL. The chromatographic analysis was run at room temperature. Chromatograms were recorded at 244 nm and were analysed by means of the LC Solution software.

Sample preparation for chromatographic analysis

About 100 mg of the mixture (prepared according to Table I) was weighed on an analytical balance, accurately. The weight was transferred quantitatively to a 100 mL volumetric flask, 50 mL of acetonitrile was added and then shaken for about 10 minutes. The

content of the flask was filtered through filter paper. 800 µL of the filtrate was transferred to a 25 mL volumetric flask, the flask was made up to the mark with a mixture of acetonitrile and water (60:40 v/v), and then mixed thoroughly in order to obtain homogenous solution. The analyte solution prepared in this way (after filtering through a syringe filter 0.22 µm) was injected onto the column of a high-performance liquid chromatograph. The exemplary chromatogram registered for hydrocortisone standard solution revealed for hydrocortisone a retention time of 3.282 min.

Results and Discussion

The application of experimental design methodology in compatibility studies seems to be promising, especially due to the lack of clearly defined guidelines regarding the method and conditions of conducting this type of studies. The applied DoE approach allows optimizing research work and consequently defining the qualitative composition of a pharmaceutical formulation. The DoE approach allows for the simultaneous assessment of the influence of several excipients on the degradation of the active substance, and consequently, the selection of optimal raw materials with a relatively small number of prepared mixtures.

In a traditionally conducted experiment, the arbitrarily determined formulation composition could omit the selection of the best excipients. The DoE methodology allows to obtain reliable information about the chemical compatibility of raw materials and their possible interactions, while maintaining the limitations imposed on the number of measurements, and thus on the cost and timeliness of studies.

Considering every possible combination of the 4 input variables, 16 different multicomponent mixtures ($2^4 = 16$) should be prepared. It would be a so-called complete (full) factorial plan. In this work, a fractional factorial plan was used. Some systems were removed from a complete plan using a special algorithm, leaving half of the initial number of mixtures. Table I presents all combinations of inputs for which hydrocortisone degradation was determined.

The collected data was analysed thoroughly. The analysis of variance (ANOVA) was performed. The results were visualized on the Pareto diagram (Figure 1), showing the effect of selected excipients on the degree of hydrocortisone degradation in the mixtures.

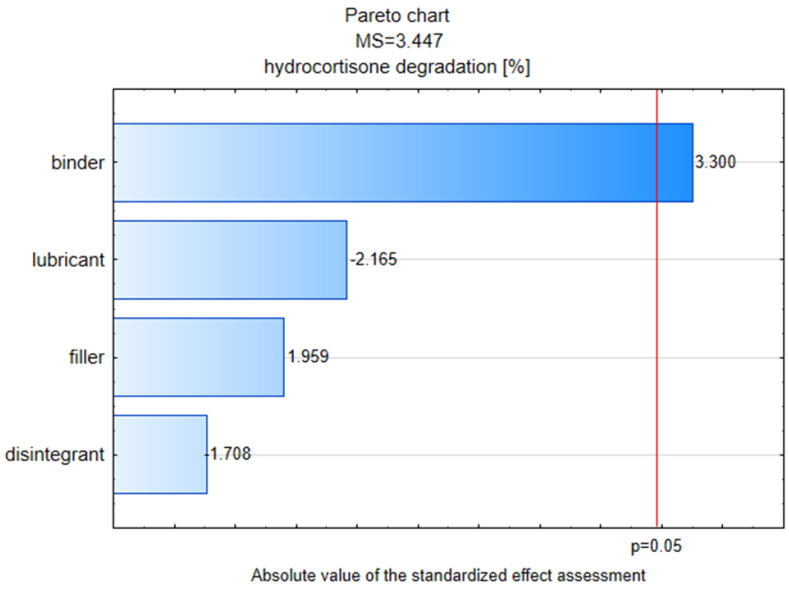

Figure 1.
Pareto chart

A chromatographic analysis of hydrocortisone content in the mixtures (after 3-month exposure to stress conditions) showed the degradation of the active pharmaceutical ingredient in all mixtures. A statistical analysis showed that the binders ($p < 0.05$) had the greatest impact on the decrease in hydrocortisone content in the mixtures.

The significant advantage of the DoE approach is a clear visualization of data and graphic interpretation of the results. The Pareto chart indicates effects with bars exceeding the critical significance level. Thus, it is possible to identify the most critical excipients, and consequently eliminate raw materials that have a negative effect on the hydrocortisone content in the formulation. The implementation of the DoE at the drug product development stage guarantees that formulation decisions are accurate and rationally justified. The analysis of the graphs (Figure 2) allows for selection the most optimal qualitative formulation composition.

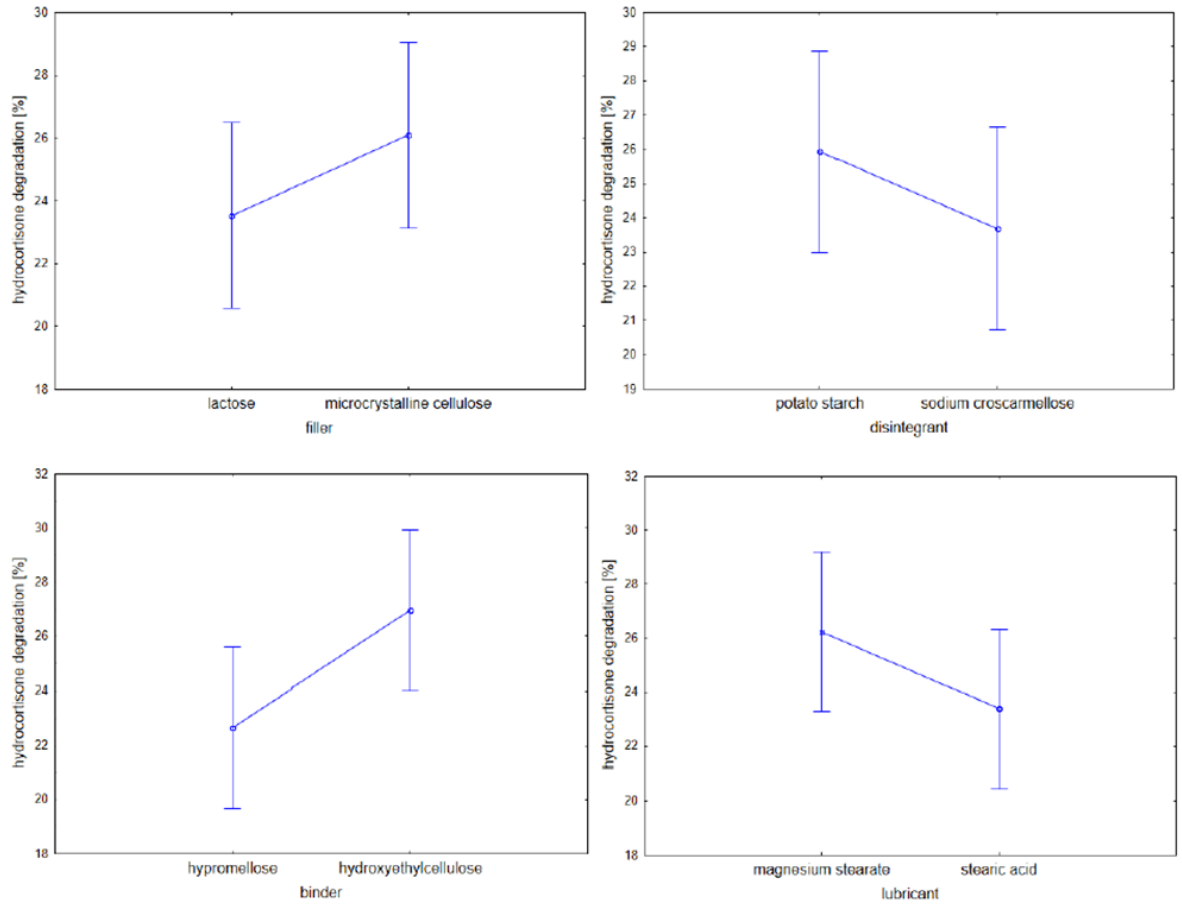

Figure 2.
Graphical interpretation of results for fillers, disintegrants, binders and lubricants

The DoE tools seem to be promising solution in order to maximize the analytical value of the collected data. The DoE methodology allows to optimize experimental research work. A non-accidental selection of a combination of input variables enables the collection of as much information as possible about the formulation with a minimum number of experiments, thus reducing the time and cost of research studies. Thanks to the DoE methodology, research work is carried out in an orderly and thought-out manner, and not in an intuitive way, using the trial and error approach [5, 11, 18, 20, 22].

The subject undertaken at this work is completely in accordance with the current trends in modern industrial pharmacy. The optimal composition of excipients for hydrocortisone was defined by assessing its degradation in all prepared mixtures after their 3-month exposure to temperature of 50°C and 50% RH humidity. The use of lactose as a filler, croscarmellose sodium as a disintegrant, hydroxypropylmethylcellulose as a binder and stearic acid as a glidant has been shown to be most advantageous.

The application of experimental design methodology at the drug product development stage allows for a better understanding of the influence of excipients on the final formulation quality. As a consequence, leading to an accurate definition of the qualitative composition of the developed formulation in a manner consistent with the *Quality by Design* concept recommended by regulatory agencies worldwide.

Conclusions

The usefulness of experimental design methodology in defining the qualitative composition of a pharmaceutical formulation was confirmed. The fractional factorial design allowed to indicate the most optimal excipients for hydrocortisone. Data from a well-designed experiment are easy to analyse. The advantage of DoE is a full statistical analysis and clear visualization of the collected data. The experimental design methodology seems to be an attractive alternative to experiments conducted in a traditional way, e.g. by trial-and-error approach. The DoE approach allows to select the most optimal formulation composition as well as it facilitates and accelerates the formulation studies, significantly. Therefore, fractional factorial design as knowledge discovery method provides useful clues for formulation scientists and thus it seems to gain more and more interest in modern pharmaceutical industry. This approach can be taken into consideration by industrial formulation scientists to support rational decision making in the field of pharmaceutical technology.

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Conflict of interest

The author declares no conflict of interest.

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