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Design of Experiments (DoE)-based approach for improvement of dry mixing processes in the production of low-dose Alprazolam tablets using Raman spectroscopy for content uniformity monitoring

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Abstract

A low-dose tablet formulation, containing a potent benzodiazepine derivative Alprazolam was developed, considering the achievement of appropriate content uniformity of the active substance in powder blends and tablets as a major challenge. Two different types of lactose monohydrate (Tablettose 80 and Granulac 200) and two different types of dry mixing processes (high-shear mixing and "in bulk" mixing) were employed. To evaluate the influence of the variables (mixing speed, mixing time, filling level of the high-shear and cube mixer, lactose monohydrate type) and their interactions upon the response (content uniformity of Alprazolam in the powder blends), a Factorial 2^4 design (with 4 factors at 2 levels in 1 block) was generated for each type of mixer. For high-shear dry mixing the Response Surface, D-optimal Factorial 2^4 design (with 2 replications and 31 experiments) was used, while for the "in bulk" dry mixing the Response Surface, Central Composite Factorial 2^4 design (with 34 experiments) was used. The process parameters for the high-shear mixer were varied within the following ranges: filling level of 70-100%, impeller mixing speed of 50-300 rpm and mixing time of 2-10 minutes. For the cube mixer the following process parameter ranges were employed: filling level of 30-60%, mixing

speed of 20-390 rpm and mixing time of 2-10 minutes. Raman spectroscopy in conjunction with a validated Partial Least Square (PLS) regression model was used as a Process Analytical Technology (PAT) tool for Alprazolam content determination and content uniformity monitoring. The DoE model was further employed to optimize the powder blending process in regard to the achievement of appropriate Alprazolam content uniformity using high-shear mixing and Tabletosse 80 as filler. The desirability function revealed that the following process parameters: a mixing time of 2 minutes, a mixing speed of 300 rpm and a 70% filling level of the mixer would produce powder blends with the lowest variability in Alprazolam content. The three independent lab batches of low-dose Alprazolam tablets, produced with high-shear mixing using these process parameters, conformed to the requirements of the European Pharmacopoeia for content (assay) of Alprazolam and uniformity of the dosage units.

Key words: Alprazolam, content uniformity, lactose monohydrate, Response Surface Factorial experimental design, Raman spectroscopy

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Introduction

The safety and efficacy of solid oral drug products, as well as their robust performance, are ensured by meeting the specified values of critical quality attributes (CQAs) among which the content uniformity (CU) of powder blends is of primary importance. Current drug product development and manufacturing should align with Quality-by-Design (QbD) principles, where formulation and processing parameters are thoroughly understood with respect to their impact on product CQAs [1]. Formulation of low-dose solid dosage forms encounters a lot of problems related to segregation, content uniformity and physical stability, which can be controlled by the appropriate selection of excipients and optimized processes. Current technological advancements have improved the manufacturing and quality of low-dose drug products by achieving their predefined objective of content uniformity during blending [2].

In general, two process-related causes of failed CU may be broadly identified: suboptimal mixing of ingredients and the failure to meet required blend uniformity or segregation of initially well-mixed material. According to Deveswaran et al., there are three types of powder blends: free-flowing mixtures, cohesive mixtures and ordered mixtures. Free-flowing powders have desirable features like a minimal need for lubricant and effective contact with the die cavity, but they have a serious drawback of segregation of individual components in post-mixing processing. The cohesive mixture components are not free-flowing, the individual particles are repeatedly broken down and allowed to redistribute within the system to ensure a satisfactorily mixed product. Ordered mixtures are formed by mechanical adhesion when the fine and micronized constituent of the powder mix is adsorbed on the larger particle's surface.

Major critical material attributes (CMAs) for achieving a homogenous blend are: mean particle size, particle size distribution, particle shape and blend flowability. The best content uniformity can be achieved if the shape, size and density of mixed particles are as similar as possible, while the spherical shape is preferred [3]. Larger particles tend to be free-flowing and smaller particles tend to be cohesive due to inter particulate forces associated with the individual particles [4].

The most important critical process parameters (CPPs) for producing homogeneous blends are: the type of mixer (blender) and the design of the mixing system (e.g. geometry and blend mechanism), blender size, fill level, rotation speed, mixing duration, blender loading mode [5]. Processing parameters are markedly influenced by the formulation parameters and require optimization for each case [6].

Although it is commonly believed that direct compression processes should be avoided for low–dose formulations because of blend and tablet content uniformity concerns, the data for direct compression processes reported by the respondents to the Product Quality Research Institute (PQRI) survey suggest that such processes are being used routinely to manufacture solid dosage forms of acceptable quality even when the drug loading is quite low. Moreover, data for direct compression processes were the most widely reported in the PQRI survey, and the blend-uniformity variation was always less than 10% [7].

There is an increasing need for methods that provide accurate and reliable information about the mixture content and homogeneity. Raman spectroscopy as a process analytical technology (PAT) tool would improve efficiency through better monitoring and control of mixing processes, resulting in fewer rejected batches and better product quality. Its applicability for continuous API content monitoring in various processing stages on the solid dosage forms has been reported in the literature [8], [9], [10], [11], [12].

The goal of this work was to optimize the dry mixing process of low-dose Alprazolam powder blends, considered a critical process in achieving CU during the manufacturing of low-dose Alprazolam tablets 1 mg. Design of Experiments (DoE) was used to evaluate the effects of the critical process and formulation parameters in two different blending processes (high-shear and cube mixer) on the CU of Alprazolam. Alprazolam content was determined using Raman spectroscopy in conjunction with multivariate analysis, a quantification model which was previously developed and optimized by our group [5]. In addition, DoE models were employed to optimize the dry mixing process and select the most appropriate excipient, which was further used to manufacture the final dosage form.

Materials and methods

Materials

Alprazolam was purchased from Centaur Pharmaceuticals (India), while the agglomerated alpha lactose monohydrate for direct compression with a trade name Tablettose®80 and milled crystalline alpha lactose monohydrate with a trade name Granulac®200 were purchased from Meggle (Germany). Crospovidone was obtained from BASF (Germany), while magnesium stearate was obtained from Mosselman (Belgium).

Methods

Particle size measurement

The particle size measurement was done with laser diffraction technique on a Mastersizer 2000 with a cell for dry dispersion Scirocco 2000A (MalvernPanalytical, UK). The measurements were carried out under the following conditions: measuring time of 5 s, dispersion pressure of 1 bar, plate vibrational intensity of 30-35% and obscuration of 1-6%. Six repeated measurements for each sample were done, and the values were expressed as average volume diameters (d10, d50 and d90) and as a diagram of a volume distribution in a range of 0.02 μm - 2000 μm.

Experimental design

To evaluate the influence of the variables (mixing speed, mixing time, filling level of the high-shear and cube mixer, lactose monohydrate type) and their interactions upon the response (content uniformity of Alprazolam in the powder

blends), a Factorial 2^4 design (with 4 factors at 2 levels in 1 block) was generated for each type of blender.

For high-shear dry mixing the Response Surface, D-optimal Factorial 2^4 design (with 2 replications and 31 experiments, Table I) was used, while the Response Surface, Central Composite Factorial 2^4 design (with 34 experiments, Table II) was employed for the “in bulk” dry mixing experiments.

High-shear dry mixing

The preparation of the binary powder blends (1% (w/w) Alprazolam and 99% (w/w) lactose) was done according to the generated DoE experiments (Table 1).

The mixing was performed in a high-shear mixer MIC 5C (Comasa, Italy) with the following process parameters: filling level was within the range of 70-100%, mixing time was varied from 2 to 10 minutes and the impeller mixing speed was within 50-300 rpm. The manufacturer recommends a working volume of approximately two thirds, i.e. 70%, from the total high-shear mixer volume. The filling level of minimum 70% enables covering the impeller and the lateral mixer (chopper) with powder that has to be mixed. The filling level within the range of 70-100% is chosen, since with lower filling level the impeller and the lateral mixer will not do their function properly. The operating speed range of the impeller is within a range from 0 to 300 rpm. The operating speed range of the lateral mixer is within a range from 300 to 3000 rpm. Based on our experience, when the powder mass is mixed at a speed lower than 50 rpm it hardly moves, so we have decided that 50 rpm should be our lower limit for the mixing speed. For dry mixing of powders in the high-shear mixer, the lateral mixer speed was kept constant in all experiments (500 rpm), due to its negligible influence in dry mixing process. The mixing speed of the lateral impeller was chosen based on experience.

Two different types of lactose monohydrate were varied in the powder blends: Tablettose 80 and Granulac 200.

With a sampling thief, 3 samples from each level of the powder blend (bottom, middle and top) were taken into a separate sampling glass container, respectively (a total of 9 samples per experiment). All the samples were further analyzed for Alprazolam content using Raman spectroscopy. RSD of Alprazolam content from the 9 collected samples was employed as a response in each mixing experiment.

Table I Factorial design for high-shear dry mixing experiments

Tabela I *Factorial design* za eksperimente suvog *high*-shear mešanja

Run	Factor 1 A: Mixing time (minutes)	Factor 2 B: Mixing speed (rpm)	Factor 3 C: Filling level of the high-shear mixer (%)	Factor 4 D: Lactose monohydrate, Type
1	6.00	175.00	85.00	Tablettose 80
2	6.00	50.00	85.00	Granulac 200
3	10.00	50.00	100.00	Tablettose 80
4	2.00	175.00	100.00	Tablettose 80
5	2.00	300.00	100.00	Granulac 200
6	6.00	112.50	85.00	Tablettose 80
7	10.00	175.00	100.00	Granulac 200
8	2.00	300.00	70.00	Tablettose 80
9	6.00	300.00	70.00	Granulac 200
10	10.00	50.00	100.00	Tablettose 80
11	6.00	300.00	85.00	Tablettose 80
12	10.00	300.00	100.00	Tablettose 80
13	2.00	175.00	70.00	Granulac 200
14	2.00	50.00	70.00	Tablettose 80
15	10.00	300.00	85.00	Granulac 200
16	10.00	50.00	70.00	Granulac 200
17	2.00	50.00	70.00	Tablettose 80
18	2.00	50.00	100.00	Granulac 200
19	6.00	300.00	70.00	Granulac 200
20	10.00	300.00	85.00	Granulac 200
21	10.00	50.00	70.00	Tablettose 80
22	2.00	50.00	100.00	Granulac 200
23	10.00	175.00	70.00	Tablettose 80
24	2.00	300.00	100.00	Granulac 200
25	8.00	175.00	77.50	Granulac 200
26	10.00	175.00	100.00	Granulac 200
27	2.00	300.00	70.00	Tablettose 80
28	10.00	300.00	100.00	Tablettose 80
29	6.00	175.00	85.00	Granulac 200
30	2.00	175.00	100.00	Tablettose 80
31	4.00	237.50	85.00	Granulac 200

"In bulk" dry mixing using a cube mixer

The powder blends were prepared according to the DoE generated table (Table II). The mixing was performed in a cube mixer KB20S (Erweka, Germany) with varying the filling level of the cube mixer (30-60%), mixing speed (20-390 rpm) and mixing time (2-10 minutes). The Cube Mixer uses a tumbling motion to produce a homogenous blend. The mixing action is assisted by three stainless steel rods, strategically positioned within the cube. The manufacturer recommends an optimal working capacity of 40% from the

total volume. We have chosen the range of 30-60% to test (challenge) the lower, recommended and higher filling volume (capacity) of the cube mixer. The operating speed range of the motor drive is 20-400 rpm.

Two different types of lactose monohydrate were varied in the binary powder blends: Tablettose 80 and Granulac 200.

Sampling was done with a sampling thief, using the same pattern as for the high-shear mixing.

Table II Factorial design for "in bulk" dry mixing experiments

Tabela II *Factorial design* za eksperimente suvog *"in bulk"* mešanja

Run	Factor 1 A: Mixing time (minutes)	Factor 2 B: Mixing speed (rpm)	Factor 3 C: Filling level of the cube mixer (%)	Factor 4 D: Lactose monohydrate, Type
1	6.00	390.00	45.00	Granulac 200
2	6.00	205.00	30.00	Granulac 200
3	8.38	315.00	36.08	Granulac 200
4	3.62	95.00	53.92	Tablettose 80
5	8.38	95.00	36.08	Granulac 200
6	6.00	205.00	45.00	Tablettose 80
7	3.62	95.00	36.08	Granulac 200
8	8.38	95.00	36.08	Tablettose 80
9	3.62	315.00	53.92	Granulac 200
10	6.00	205.00	45.00	Granulac 200
11	8.38	95.00	53.92	Granulac 200
12	6.00	205.00	45.00	Granulac 200
13	6.00	205.00	45.00	Tablettose 80
14	6.00	20.00	45.00	Tablettose 80
15	3.62	315.00	53.92	Tablettose 80
16	10.00	205.00	45.00	Tablettose 80
17	6.00	390.00	45.00	Tablettose 80
18	2.00	205.00	45.00	Granulac 200
19	8.38	315.00	53.92	Granulac 200
20	6.00	205.00	30.00	Tablettose 80
21	6.00	205.00	60.00	Granulac 200
22	3.62	95.00	53.92	Granulac 200
23	8.38	95.00	53.92	Tablettose 80
24	6.00	20.00	45.00	Granulac 200
25	2.00	205.00	45.00	Tablettose 80
26	10.00	205.00	45.00	Granulac 200

Run	Factor 1 A: Mixing time (minutes)	Factor 2 B: Mixing speed (rpm)	Factor 3 C: Filling level of the cube mixer (%)	Factor 4 D: Lactose monohydrate, Type
27	3.62	315.00	36.08	Tablettose 80
28	8.38	315.00	36.08	Tablettose 80
29	6.00	205.00	60.00	Tablettose 80
30	6.00	205.00	45.00	Tablettose 80
31	8.38	315.00	53.92	Tablettose 80
32	6.00	205.00	45.00	Granulac 200
33	3.62	315.00	36.08	Granulac 200
34	3.62	95.00	36.08	Tablettose 80

The sampling and Alprazolam content analysis were performed as previously described.

Raman spectroscopy

The Alprazolam content was determined using Raman spectroscopy on JobinYvon LabRam300 Infinity (Horiba, France) in conjunction with a validated Partial Least Square (PLS) regression model, as described in our previous work [5]. A 532 nm Nd:YAG frequency doubled laser without the use of a filter (no laser photodegradation was obtained) was used as a light source, while a ×50 long-distance objective was selected from an Olympus MPlanN confocal microscope. Raman mapping of 6x6 spectral points was performed on a 5x5 mm^2 area from each sample. The Alprazolam content was calculated by the validated PLS model, while some of the samples were additionally analyzed using HPLC, to periodically test the prediction capability of the PLS model.

HPLC method for Alprazolam content and content uniformity analysis

HPLC was used as the reference method for Alprazolam content and CU determination to verify the statistical error indicators of the Raman spectroscopy based PLS model. The HPLC method is described in our other article [5]. In brief, the analyses were performed on UHPLC NEXERA Lab Solution-2ch (Shimadzu, Japan) using LiChrospher RP Select B (5 μm), 125 x 4 column (MerckMilipore, Germany), flow rate of 1.4 mL/min, column temperature at 30°C, and an injection volume of 20 μL. DAD detector at a wavelength of 220 nm was used. The mobile phase was prepared from methanol-acetonitrile potassium dihydrogen phosphate buffer (pH 3.0; 15 mM) (10:45:45, v/v/v). The pH of the potassium dihydrogen phosphate buffer was adjusted using o-phosphoric acid. The Alprazolam content was calculated on the basis of Alprazolam peak areas, relative to the standard calibration curve.

Preparation of the Alprazolam tablets with the optimized formulation and process parameters

The optimal formulation/dry mixing process selected by the DoE was further used for preparation of three lab batches of Alprazolam. The prepared powder blend was characterized in terms of flowability and Carr's index (European Pharmacopeia) and afterwards compressed using 7 t/cm^2 on the automatic laboratory tablet press MiniPress-2B (Pharmag Maschinen und Gerätebau, Germany), producing tablets with a diameter of 7 mm and mass of 130 mg. The tablets were characterized by average weight and weight variation, as well as in CU according to the appropriate European Pharmacopeia monographs.

Results and discussion

Particle size and morphology

Particle size and particle size distribution determined by laser diffractometry are shown in Table III and Figure 1.

Table III Particle size and particle size distribution of Alprazolam and two types of lactose monohydrate - Tablettose 80 and Granulac 200

Tabela III Veličina čestica i distribucija čestica po veličini Alprazolama i dva tipa Laktoze monohidrata - Tablettose 80 and Granulac 200

Substance name	Average value of results obtained			
	d (0.1) (µm)	d (0.5) (µm)	d (0.9) (µm)	Span
Alprazolam	1.096±0.03	3.113±0.07	6.469±0.35	1.77±0.08
Tablettose 80	32.583±0.76	126.269±4.32	330.088±8.74	2.35±0.07
Granulac 200	6.247±0.36	35.939±1.68	109.97±4.03	2.88±0.03

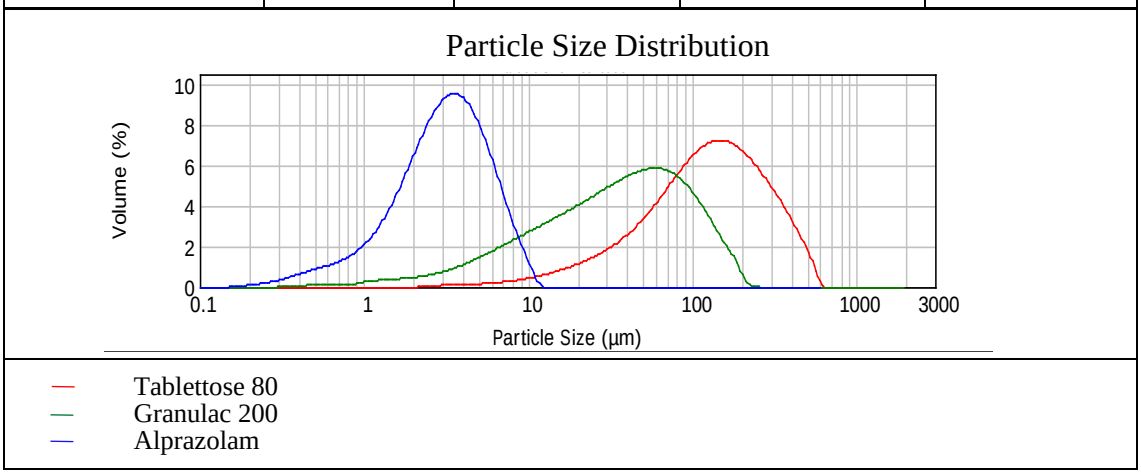


Figure 1. Particle size and particle size distribution (PSD) of Alprazolam and two types of lactose monohydrate - Tablettose 80 and Granulac 200

Slika 1. Veličina čestica i distribucija čestica po veličini (PSD) Alprazolama i dva tipa Laktoze monohidrata - Tablettose 80 and Granulac 200

The Alprazolam sample is clearly distinguished with the smallest particles, compared to those of Granulac 200 and Tablettose 80. The largest are the particles of Tablettose 80. In terms of the particle size distribution (PSD), Alprazolam particles have an almost normal distribution i.e., they closely follow the Gaussian function of normal distribution. Granulac 200 and Tablettose 80 show an asymmetry in PSD. The left region of the distribution curves (representing the smaller particle sizes) in both types of lactose monohydrate is distributed over a wider range, and the larger particles are distributed in a narrower range. It can be noted that the particles of Granulac 200 have a significantly lower mean volume diameter than those of Tablettose 80. Moreover, according to the manufacturer's specifications, in addition to the differences in diameters of particles, these lactose monohydrate types differ in their morphological characteristics.

Tablettose 80 is an alpha agglomerated lactose monohydrate, intended for direct compression. According to the manufacturer’s specifications, the particles are larger and round, with rough structured surfaces [13]. Due to its characteristic shape and particle morphology, Tablettose 80 provides stable, homogenous powder blends with other excipients and APIs.

On the other hand, the Granulac 200 is a milled crystalline alpha lactose monohydrate that exhibit different morphology. The milled crystalline lactose monohydrate grades consist of fine, smaller lactose particles with disrupted and sharp edges [13]. The milled alpha lactose monohydrate shows a limited flowability, and these grades have been historically used as diluents (fillers) in dry and wet granulation processes [14].

Raman spectroscopy model validation

The Raman spectroscopy model validation was performed with a validation series of 22 samples with concentrations of Alprazolam that span in a broader concentration range (0.1–2.2%), that were randomly selected from both DoE experiments. The concentration of Alprazolam in each sample was determined by HPLC method and calculated from the first derivative of their Raman spectrum in the range of 700–1700 cm-1 using the optimized calibration model. The results are shown in Figure 2.

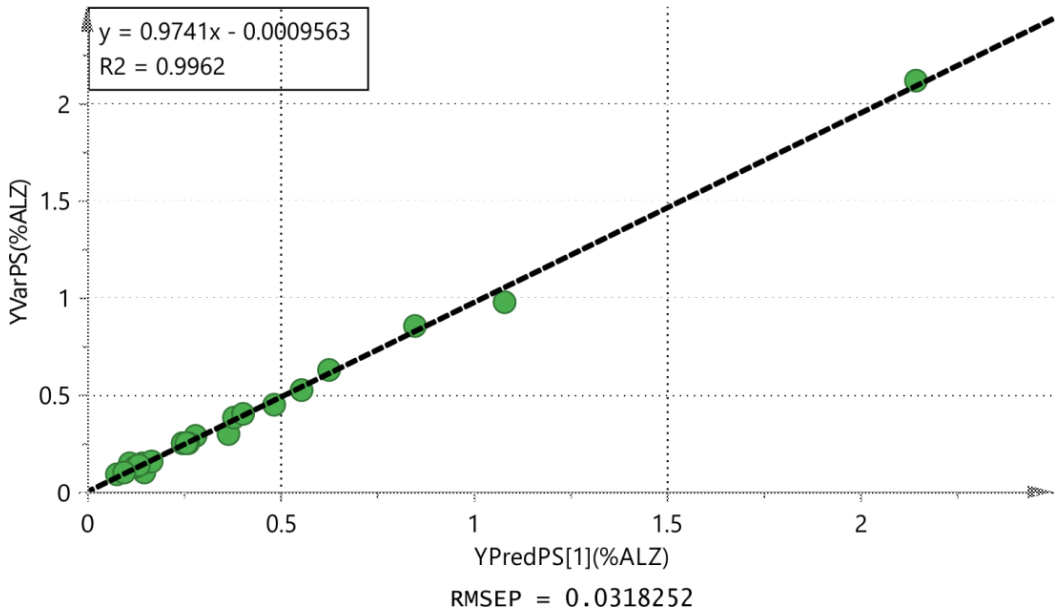


Figure 2. **Graph of the observed vs. predicted Y data for Alprazolam (ALZ) concentration in the validation samples (22 validation samples)**

Slika 2. **Grafikon dobijenih naspram predviđenih Y podataka za koncentraciju Alprazolama (ALZ) u validacionim uzorcima (22 validacioni uzorci)**

The graph of the observed (HPLC) vs. predicted (Raman) data for Alprazolam content in the validation series of samples shows that the scattering of the points is negligible, and the regression coefficient is 0.996. Root Mean Square Error of Prediction (RMSEP) is 0.032%, which is an appropriate value for the expected range of Alprazolam concentrations in dry powder blends (mixtures) to be examined during the evaluation and optimization of dry mixing processes.

Experimental design for the high-shear mixing process

To investigate and quantify the influence of the independent variables: process parameters (mixing time, mixing speed and filling level of the high-shear mixer) and the particle size and particle morphology of the filler lactose monohydrate upon the Alprazolam CU in the powder blend, an experimental design with Response Surface Methodology (RSM) was used. The experiments were performed based on the previously specified matrix. The results for the Alprazolam content relative standard deviation (AC-RSD) at different points in the binary powder blend, determined by the validated Raman spectroscopy method, are shown in Table IV.

Table IV Actual values and responses for the Experimental factorial 2^4 design for high-shear dry mixing

Tabela IV Realne vrednosti i odgovori za *Experimental factorial 2^4 design* za *high-shear* suvo mešanje

Run	Block	Factor 1 Mixing time (minutes)	Factor 2 Mixing speed (rpm)	Factor 3 Filling level of the high-shear mixer (%)	Factor 4 Lactose monohydrate, Type	Response 1 AC-RSD (%)
1	Block 1	6.00	175.00	85.00	Tablettose 80	2.698
2	Block 1	6.00	50.00	85.00	Granulac 200	8.53
3	Block 1	10.00	50.00	100.00	Tablettose 80	3.506
4	Block 1	2.00	175.00	100.00	Tablettose 80	6.6
5	Block 1	2.00	300.00	100.00	Granulac 200	12.69
6	Block 1	6.00	112.50	85.00	Tablettose 80	4.072
7	Block 1	10.00	175.00	100.00	Granulac 200	4.144
8	Block 1	2.00	300.00	70.00	Tablettose 80	3.106
9	Block 1	6.00	300.00	70.00	Granulac 200	2.306
10	Block 1	10.00	50.00	100.00	Tablettose 80	3.72677
11	Block 1	6.00	300.00	85.00	Tablettose 80	3.882
12	Block 1	10.00	300.00	100.00	Tablettose 80	8.19
13	Block 1	2.00	175.00	70.00	Granulac 200	7.724
14	Block 1	2.00	50.00	70.00	Tablettose 80	4.188
15	Block 1	10.00	300.00	85.00	Granulac 200	3.672
16	Block 1	10.00	50.00	70.00	Granulac 200	3.082
17	Block 1	2.00	50.00	70.00	Tablettose 80	5.6582
18	Block 1	2.00	50.00	100.00	Granulac 200	13.5
19	Block 1	6.00	300.00	70.00	Granulac 200	5.91999
20	Block 1	10.00	300.00	85.00	Granulac 200	0.946811
21	Block 1	10.00	50.00	70.00	Tablettose 80	5.476
22	Block 1	2.00	50.00	100.00	Granulac 200	8.818
23	Block 1	10.00	175.00	70.00	Tablettose 80	2.606
24	Block 1	2.00	300.00	100.0	Granulac 200	10.4749
25	Block 1	8.00	175.00	77.50	Granulac 200	3.236
26	Block 1	10.00	175.00	100.00	Granulac 200	0.718577
27	Block 1	2.00	300.00	70.00	Tablettose 80	3.96353
28	Block 1	10.00	300.00	100.00	Tablettose 80	5.37783
29	Block 1	6.00	175.00	85.00	Granulac 200	3.594
30	Block 1	2.00	175.00	100.00	Tablettose 80	5.00319
31	Block 1	4.00	237.50	85.00	Granulac 200	8.438

The analysis of variance (ANOVA) and the statistical indicators of the model are shown in Table V.

Table V ANOVA and the statistical indicators of the high-shear dry mixing model

Tabela V ANOVA i statistički indikatori modela *high-shear* suvog mešanja

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	235.62	10	23.56	8.10	< 0.0001
A-Mixing time	83.86	1	83.86	28.82	< 0.0001
B-Mixing speed	0.5962	1	0.5962	0.2049	0.6557
C-Filling level of the high-shear mixer	14.14	1	14.14	4.86	0.0394
D-Lactose monohydrate, Type	17.06	1	17.06	5.86	0.0251
AB	0.2529	1	0.2529	0.0869	0.7712
AC	0.6697	1	0.6697	0.2301	0.6366
AD	64.33	1	64.33	22.11	0.0001
BC	14.29	1	14.29	4.91	0.0385
BD	2.95	1	2.95	1.01	0.3260
CD	0.1440	1	0.1440	0.0495	0.8262
Residual	58.20	20	2.91		
Lack of Fit	21.97	10	2.20	0.6065	0.7785
Pure Error	36.23	10	3.62		
Cor Total	293.82	30			

Std. Dev.	1.71	**R^2**	0.8019
Mean	5.35	**Adjusted R^2**	0.7029
C.V. %	31.89	**Predicted R^2**	0.5187
		Adequate Precision	9.5467

According to statistical indicators, it can be noted that the model is significant and can explain the relations among the independent and dependent variables, with a high degree of correlation and predictability. In addition, the quadratic interactions do not have the appropriate statistical significance, which excludes them from the model evaluation phase. The factors: mixing time, filling level of the high-shear mixer and lactose monohydrate (filler) type, as well as the interactions: mixing time-lactose monohydrate type and mixing speed-filling level of the high-shear mixer have a significant statistical influence on the Alprazolam CU in the powder blend (Figure 3). The equation (Eq. 1) for calculating the AC-RSD when Tablettose 80 is used as filler is:

AC-RSD, high-shear mixing with Tablettose 80 *= 6.80779 + 0.17355 * mixing time - 0.045242 * mixing speed - 0.022073 * filling level of high-shear mixer + 2.63946E-004 * mixing time * mixing speed - 3.40528E-003 * mixing time * filling level of high-shear mixer+ 5.34102E-004 * mixing speed * filling level of high-shear mixer* (1)

If Granulac 200 is used as filler, according to the model, the equation (Eq. 2) can be written as:

AC-RSD, high-shear mixing with Granulac 200 *= 13.98354 - 0.73458 * mixing time - 0.051534 * mixing speed - 0.01061 * filling level of high-shear mixer + 2.63946E-004 * mixing time * mixing speed - 3.40528E-003 * mixing time * filling level of high-shear mixer + 5.34102E-004 * mixing speed * filling level of high-shear mixer* *(2)*

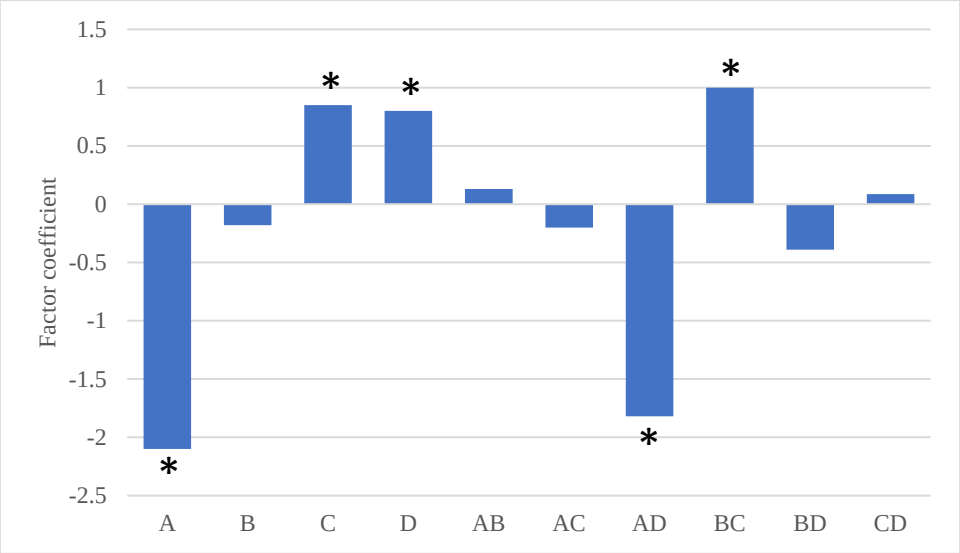
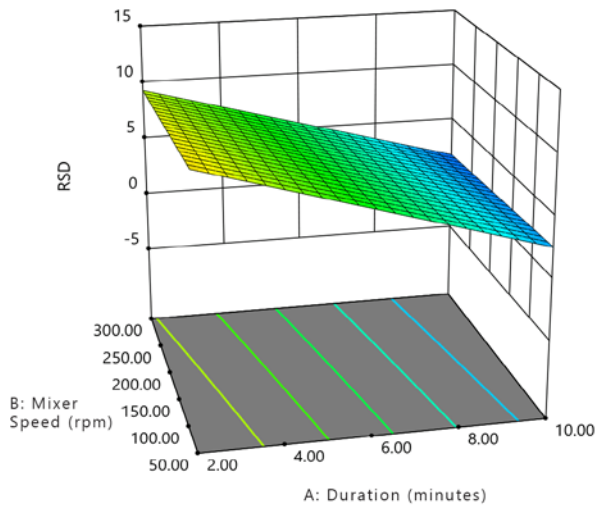


Figure 3. Coded effects of examined factors and their interactions in high-shear dry mixing DoE; A-Mixing time, B-Mixing speed, C-Filling level of the high-shear mixer, D-Lactose monohydrate type; the asterix denominates statistically significant effects (p<0.05)

Slika 3. Kodirani efekti ispitanih faktora i njihovih interakcija za DoE *high-shear* suvog mešanja; A-Vreme mešanja, B-Brzina mešanja, C-Nivo punjenja *high-shear* miksera, tip D-Lactose monohydrate; zvezdica denominira statistički značajne efekte (p<0.05)

From the DoE statistical indicators, it is evident that, when using Tablettose 80 as filler, increasing the mixing time will result in higher AC-RSD, and the increase of filling level of the high-shear mixer will produce lower AC-RSD, even though the later effect (filling level) is weaker. Differences in the influence of the factors on the Alprazolam CU

in the powder blend, when using different types of lactose monohydrate, are due to particle size, particle size distribution and particle morphology of the different types of lactose monohydrate. Tablettose 80 features larger particles with spherical morphology, thus having better flowability characteristics than Granulac 200, which has particles of a smaller diameter and crystalline morphology with very sharp edges. When mixing with high-shear forces, due to the large contact surface, irregular morphology of the crystals of Granulac 200 and adhesion of micronized active substance particles on the filler surface may occur. The adhered active substance will not be able to segregate during the process and when the dry mixing is finished. In the case of Tablettose 80, however, given the robust characteristics in terms of flowability and particle integrity, the mixing time has less impact on the active substance content uniformity in the powder blend. According to the coefficient values, it can be concluded that in the case of Tablettose 80 prolonged mixing is likely to lead to the segregation of the binary powder blend, which was not the case when Granulac 200 was used as filler. The filling level of the high-shear mixer in both cases (Tablettose 80 and Granulac 200) has less influence than the mixing time and AC-RSD tends to get higher when lower filling levels are employed, in the filling level range tested (Figures 4 and 5). However, it can be noted that the influence of the high-shear mixer filling level is twice as large when Tablettose 80 is used, which can be explained again by differences in particle size, particle size distribution and particle morphology compared to Granulac 200. The mixing speed factor, according to the model, had no significant influence in both cases (when using Tablettose 80 and when using Granulac 200).

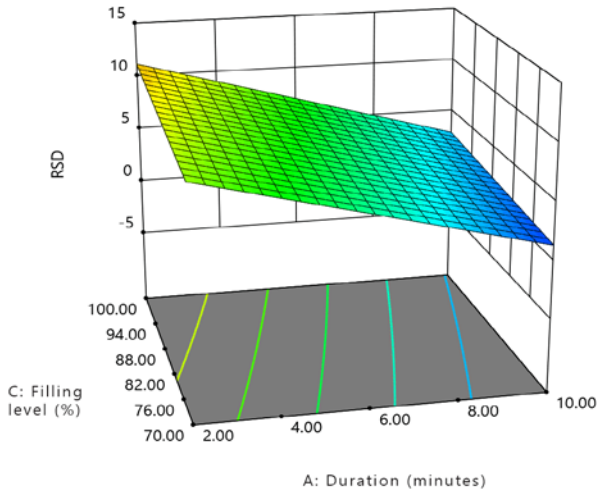


A: Mixing time (minutes)

B: Mixing Speed (rpm)

C: Filling level of the high-shear mixer (85%)

D: Lactose monohydrate, Type (Granulac 200)



A: Mixing time (minutes)

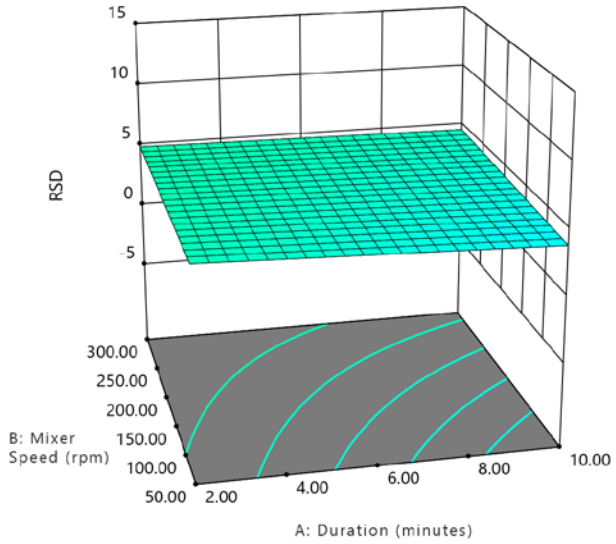
B: Mixing Speed (180 rpm)

C: Filling level of the high-shear mixer (%)

D: Lactose monohydrate, Type (Granulac 200)

Figure 4. **3D diagrams of the dependence of the AC-RSD in the binary powder blend with Granulac 200 on a) mixing time and mixing speed (with 85% filling level of the high-shear mixer) and b) mixing time and filling level of the high-shear mixer (with a mixing speed of 180 rpm)**

Slika 4. **3D dijagrami zavisnosti AC-RSD binarne praškaste mešavine sa Granulac-om 200 od a) vremena mešanja i brzine mešanja (85% nivo punjenja *high-shear* miksera) i b) vremena mešanja i nivoa punjenja *high-shear* miksera (brzina mešanja 180 rpm)**

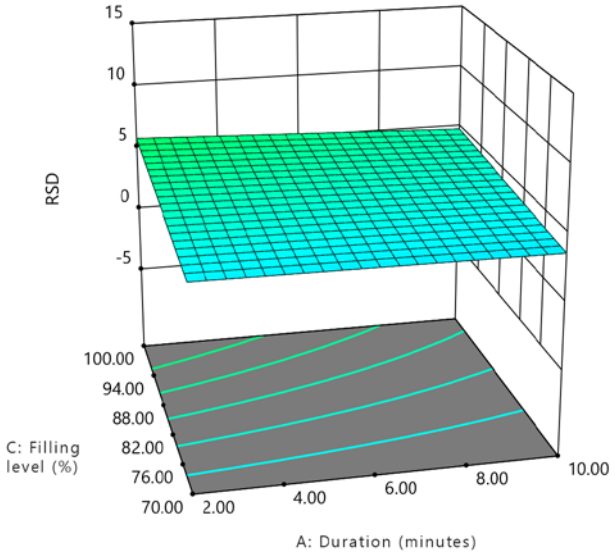


A: Mixing time (minutes)

B: Mixing speed (rpm)

C: Filling level of the high-shear mixer (85%)

D: Lactose monohydrate, Type (Tablettose 80)



A: Mixing time (minutes)

B: Mixing Speed (180 rpm)

C: Filling level of the high-shear mixer (%)

D: Lactose monohydrate, Type (Tablettose 80)

Figure 5. **3D diagrams of the dependence of the AC-RSD in the binary powder blend with Tablettose 80 on a) mixing time and mixing speed (with 85% filling level of the high-shear mixer) and b) mixing time and filling level of the high-shear mixer (with a mixing speed of 180 rpm)**

Slika 5. **3D dijagrami zavisnosti AC-RSD binarne praškaste mešavine sa Tablettose-om 80 od a) vremena mešanja i brzine mešanja (85% nivo punjenja *high-shear* miksera) i b) vremena mešanja i nivoa punjenja *high-shear* miksera (brzina mešanja 180 rpm)**

Experimental design for the preparation of binary powder blends with an "in bulk" mixing process

The results for the AC-RSD at different points in the binary powder blend, determined by the validated Raman spectroscopy method are shown in the Table VI.

Table VI Actual values and responses for the Experimental factorial 2^4 design for "in bulk" dry mixing

Tabela VI Realne vrednosti i odgovori za *Experimental factorial 2^4 design* za "in bulk" suvo mešanje

Run	Block	Factor 1 Mixing time (minutes)	Factor 2 Mixing speed (rpm)	Factor 3 Filling level of the cube mixer (%)	Factor 4 Lactose monohydrate, Type	Response 1 AC-RSD (%)
1	Block 1	6.00	390.00	45.00	Granulac 200	31.2146
2	Block 1	6.00	205.00	30.00	Granulac 200	24.4655
3	Block 1	8.38	315.00	36.08	Granulac 200	33.2765
4	Block 1	3.62	95.00	53.92	Tablettose 80	43.5514
5	Block 1	8.38	95.00	36.08	Granulac 200	43.9558
6	Block 1	6.00	205.00	45.00	Tablettose 80	17.4774
7	Block 1	3.62	95.00	36.08	Granulac 200	55.0311
8	Block 1	8.38	95.00	36.08	Tablettose 80	6.07625
9	Block 1	3.62	315.00	53.92	Granulac 200	31.1053
10	Block 1	6.00	205.00	45.00	Granulac 200	37.6616
11	Block 1	8.38	95.00	53.92	Granulac 200	45.8102
12	Block 1	6.00	205.00	45.00	Granulac 200	41.0881
13	Block 1	6.00	205.00	45.00	Tablettose 80	16.2772
14	Block 1	6.00	20.00	45.00	Tablettose 80	44.1025
15	Block 1	3.62	315.00	53.92	Tablettose 80	24.5682
16	Block 1	10.00	205.00	45.00	Tablettose 80	4.47404
17	Block 1	6.00	390.00	45.00	Tablettose 80	10.0563
18	Block 1	2.00	205.00	45.00	Granulac 200	33.1
19	Block 1	8.38	315.00	53.92	Granulac 200	23.9279
20	Block 1	6.00	205.00	30.00	Tablettose 80	15.0187
21	Block 1	6.00	205.00	60.00	Granulac 200	43.7
22	Block 1	3.62	95.00	53.92	Granulac 200	62.1892
23	Block 1	8.38	95.00	53.92	Tablettose 80	4.67418
24	Block 1	6.00	20.00	45.00	Granulac 200	39.4687
25	Block 1	2.00	205.00	45.00	Tablettose 80	23.1256
26	Block 1	10.00	205.00	45.00	Granulac 200	42.4677
27	Block 1	3.62	315.00	36.08	Tablettose 80	29.6267
28	Block 1	8.38	315.00	36.08	Tablettose 80	9.67351
29	Block 1	6.00	205.00	60.00	Tablettose 80	11.4722
30	Block 1	6.00	205.00	45.00	Tablettose 80	13.9375
31	Block 1	8.38	315.00	53.92	Tablettose 80	1.78484
32	Block 1	6.00	205.00	45.00	Granulac 200	26.0419
33	Block 1	3.62	315.00	36.08	Granulac 200	45.75
34	Block 1	3.62	95.00	36.08	Tablettose 80	43.808

The analysis of variance (ANOVA) and the statistical indicators of the model are shown in Table VII.

Table VII ANOVA and the statistical indicators of the "in bulk" dry mixing model

Tabela VII ANOVA i statistički indikatori modela "in bulk" suvog mešanja

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Model	6572.60	10	657.26	8.64	< 0.0001
A-Mixing time	1213.58	1	1213.58	15.95	0.0006
B-Mixing speed	1140.84	1	1140.84	15.00	0.0008
C-Filling level of the cube mixer	0.3756	1	0.3756	0.0049	0.9446
D-Lactose monohydrate, Type	3411.00	1	3411.00	44.84	< 0.0001
AB	108.56	1	108.56	1.43	0.2444
AC	0.9917	1	0.9917	0.0130	0.9101
AD	521.62	1	521.62	6.86	0.0154
BC	122.62	1	122.62	1.61	0.2169
BD	0.3094	1	0.3094	0.0041	0.9497
CD	52.69	1	52.69	0.6927	0.4138
Residual	1749.65	23	76.07		
Lack of Fit	1618.79	19	85.20	2.60	0.1827
Pure Error	130.86	4	32.72		
Cor Total	8322.25	33			

Std. Dev.	8.72	**R^2**	0.7898
Mean	28.82	**Adjusted R^2**	0.6984
C.V. %	30.26	**Predicted R^2**	0.5006
		Adequate Precision	11.1070

The statistical indicators (Table VII), point towards the model's capability for explaining the relations among independent and dependent variables, with a high degree of correlation and predictability. In this case, as in the previous one, the quadratic interactions do not have a statistically significant influence and they were excluded from the model evaluation phase. The factors: mixing time, mixing speed and lactose monohydrate (filler) type, as well as the interaction mixing time-lactose monohydrate type have a statistically significant influence on the Alprazolam CU in the powder blend (Figure 6). The equation (Eq. 3) of the model for calculating the AC-RSD when Tablettose 80 is used as filler is:

AC-RSD, "in bulk" dry mixing with Tablettose 80 *= 49.53473- 6.15279 * mixing time + 7.52147E-003 * mixing speed + 0.47999 * filling level of the cube mixer + 9.95587E-003 * mixing time * mixing speed - 0.011736 * mixing time * filling level of the cube mixer - 2.82170E-003 * mixing speed * filling level of the cube mixer (3)*

When Granulac 200 is used as filler, according to the model, the equation (Eq. 4) is:

AC-RSD, "in bulk" dry mixing with Granulac 200 *= 33.10607 - 2.47801 * mixing time + 9.45652E-003 * mixing speed + 0.79145 * filling level of the cube mixer + 9.95587E-003 * mixing time * mixing speed - 0.011736 * mixing time * filling level of the cube mixer - 2.82170E-003 * mixing speed * filling level of the cube mixer (4)*

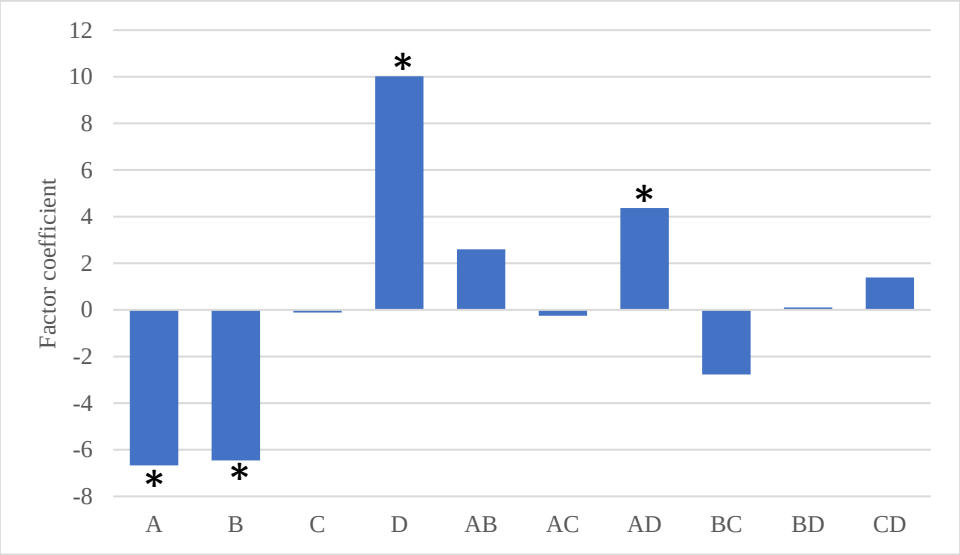
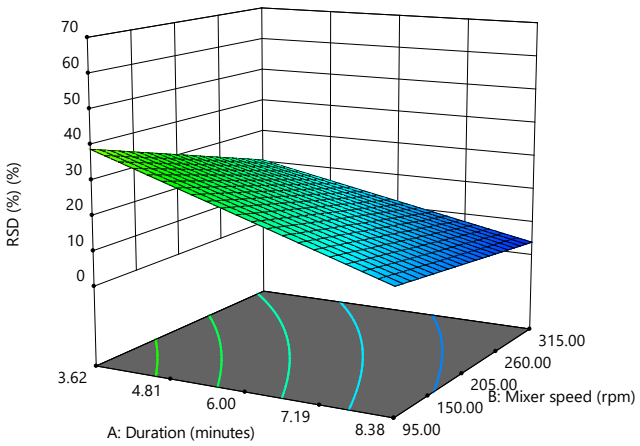


Figure 6. Coded effects of the examined factors and their interactions in the "in bulk" dry mixing DoE; A-Mixing time, B-Mixing speed, C-Filling level of the high-shear mixer, D-Lactose monohydrate type; the asterix denominates statistically significant effects (p<0.05)

Slika 6. Kodirani efekti ispitanih faktora i njihovih interakcija za DoE *"in bulk"* suvog mešanja; A-Vreme mešanja, B-Brzina mešanja, C-Nivo punjenja *"in bulk"* miksera, tip D-Lactose monohydrate; zvezdica denominira statistički značajne efekte (p<0.05)

In the case of "in bulk" dry mixing using a cube mixer, as mixing time is increased the AC-RSD in binary powder blend decreases (Figure 7a and b). The coefficient of this factor when using Tablettose 80 is higher, primarily due to its morphological characteristics and flowability. The flowability of the particles during "in bulk" dry mixing is one of the major factors affecting the powder blend uniformity, thus affecting Alprazolam CU in the

powder blend. Therefore, the mixing time is expected to have a greater effect on the filler with better flowability characteristics. The mixing speed factor in the case of “in bulk” dry mixing has a statistically significant effect on the AC-RSD. The increase of the mixing speed, in both cases (Tablettose 80 and Granulac 200), leads to non-uniform mixing due to the possible adherence of micronized Alprazolam particles to the cube mixer wall, thus increasing the AC-RSD in the powder blend. The filling level of the cube mixer in both cases (Tablettose 80 and Granulac 200) has no significant effect in the tested range.

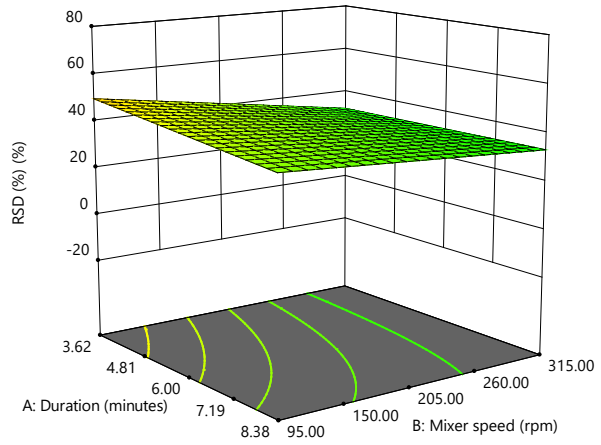


A: Mixing time (minutes)

B: Mixing speed (rpm)

C: Filling level of the cube mixer (45%)

D: Lactose monohydrate, Type (Tablettose 80)



A: Mixing time (minutes)

B: Mixing speed (rpm)

C: Filling level of the cube mixer (45%)

D: Lactose monohydrate, Type (Granulac 200)

Figure 7. 3D diagrams of the: a) dependence of the AC-RSD in the binary powder blend with Tablettose 80 on mixing time and mixing speed (with 45% filling level of the cube mixer) and b) dependence of the AC-RSD in the binary powder blend with Granulac 200 on mixing time and mixing speed (with 45% filling level of the cube mixer)

Slika 7. 3D dijagrami a) zavisnosti AC-RSD binarne praškaste mešavine sa Tablettose-om 80 od vremena mešanja i brzine mešanja (45% nivo punjenja kockastog miksera) i b) zavisnosti AC-RSD binarne praškaste mešavine sa Granulac-om 200 od vremena mešanja i brzine mešanja (45% nivo punjenja kockastog miksera)

Optimization of the process of dry mixing

According to the experimental designs of the two dry mixing methods, it was decided to further continue with an optimization of the high-shear dry mixing method only, due to the evident differences between the results for AC-RSD. The AC-RSD range in the high-shear dry mixing experiments (0.72-13.5% and 2.6-8.6% for Granulac 200 and Tablettose 80, respectively) was within the limits of tolerance for this type of dosage form, which was not the case for the "in bulk" dry mixing, where large AC-RSD values (23.9-62.2% and 1.8-44.1%, for Granulac 200 and Tablettose 80, respectively) unsuitable for this type of dosage form were obtained. Moreover, due to the poor flowability and compressibility, the lactose monohydrate Granulac 200 was excluded from the further optimization phase.

Numerical optimization of the high-shear dry mixing process was performed. There were no restrictions for calculating the optimal process parameters (equal use of all parameters according to the design area was allowed). The optimization aimed towards reducing the values of the response (AC-RSD), i.e., obtaining a powder blend of Alprazolam and lactose monohydrate (Tablettose 80) with appropriate Alprazolam content uniformity. The following experiments were obtained (Table VIII) with calculated Desirability Index or D function (Desirability Function).

Table VIII Experiments with calculated D function (Desirability Function)
Tabela VIII Eksperimenti sa izračunatom D funkcijom (Desirability Function)

No	Mixing time (minutes)	Mixing speed (rpm)	Filling level of high-shear mixer (%)	Lactose monohydrate, Type	AC-RSD	D function (Desirability Function)
1	**2.000**	**300.000**	**70.000**	**Tablettose**	**2.935**	**0.827**
2	2.000	299.999	70.000	Tablettose	2.935	0.827
3	2.368	300.000	70.000	Tablettose	2.940	0.826
4	2.000	299.079	70.000	Tablettose	2.942	0.826
5	2.117	300.000	70.040	Tablettose	2.942	0.826

From the cited values (Table VIII) it can be concluded that optimal conditions of high-shear dry mixing of Alprazolam with Tablettose 80 are achieved at a low filling level of the high-shear mixer, maximum mixing speed and short mixing time. The dependence of the D function and AC-RSD on the mixing speed and mixing time at the lowest filling level of high-shear mixer (70%) is shown in Figure 8 (left and right).

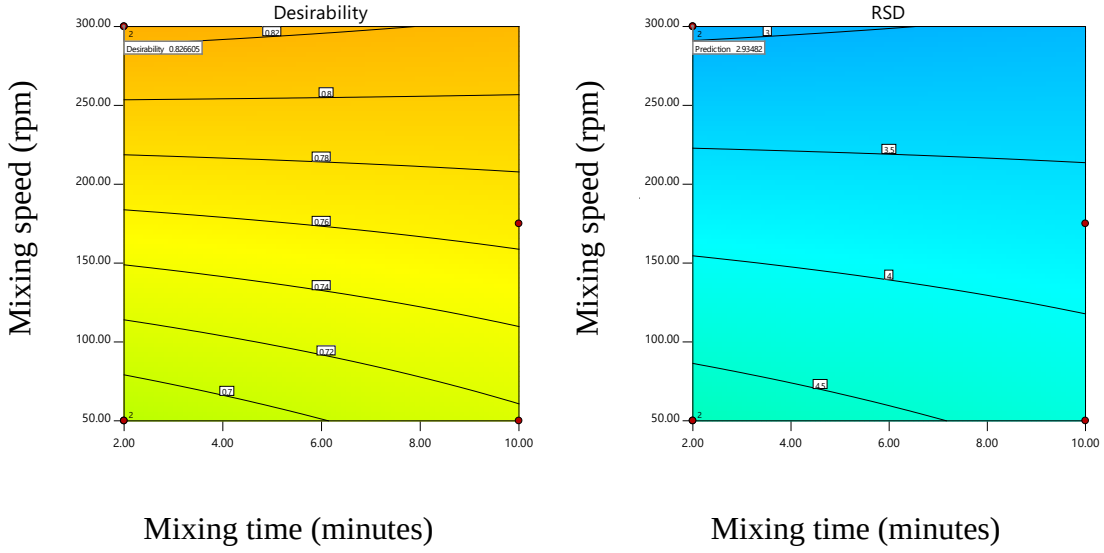


Figure 8. Contour diagrams of the dependence of the D function (left) and the dependence of the AC-RSD (right) on the mixing time and mixing speed of Alprazolam and Tablettose 80 in a high-shear mixer filled to a level of 70%

Slika 8. Konturni dijagrami zavisnosti D funkcije (levo) i zavisnosti AC-RSD (desno) od vremena mešanja i brzine mešanja Alprazolama i Tablettose 80 u *high-shear* mikserom (nivo punjennja 70%)

The cross-validation of the model (performed with three replications of the optimal batch) resulted in an AC-RSD of 3.12±0.33% in the powder blends and 1.49% standard error of prediction of the model.

Preparation of Alprazolam tablets

The optimal method of high-shear dry mixing was further used for the preparation of three lab-scale batches of Alprazolam tablets. The active substance Alprazolam was mixed with Tablettose 80 in the high-shear mixer for 2 minutes, with a mixing speed of 300 rpm and 70% filling level of the high-shear mixer, where crospovidone (2%) was also added into the powder blend. The lubricant magnesium stearate (1.2%) was added in the next phase and additionally mixed for 0.5 minutes, with a mixing speed of 180 rpm. The flowability of 7 g/s (through an orifice with a diameter of 11.3 mm) and Carr's index of 16 indicated the free-flowing characteristics of the powder blend. The powder blend was then compressed into uniform, round, biconvex tablets with a score line on one side and a diameter of 7 mm. The quality control of the final tablets revealed that all lab batches conformed to the requirements and limits of tolerance for the parameters given in the specification of quality in accordance with the relevant monographs of the latest valid European Pharmacopoeia (Table IX).

Table IX Specification and Certificate of Quality for three lab scale batches of low dose Alprazolam tablets

Tabela IX Specifikacija i Sertifikat Kvaliteta za tri laboratorijske serije tableta sa niskom dozom Alprazolama

Parameter	**Requirements / Limits of Tolerance**	**Lab scale batch 1**	**Lab scale batch 2**	**Lab scale batch 3**
Description:	Uniform, round, biconvex tablets with a score line on one side			
Average mass: (Ph. Eur., 2.9.5.)	130 mg±7.5 %	130.45 mg	130.31 mg	128.94 mg
Mass variation: (Ph.Eur., 2.9.5.)	max±7.5 %, the most 2 out of 20 tablets±15.0 %	+ 1.11% - 0.88%	+ 0.99% - 0.78%	+ 0.98% - 0.81%
Assay: - HPLC	0.95 mg-1.05 mg Alprazolam/ tablet or 95-105 % of the declared content	103.40%	98.57%	97.21%
Uniformity of dosage unit (tablet): (Ph.Eur., 2.9.40.)	Acceptance Value (AV) ≤ 15.0 (L1)	7.21	4.59	3.79

Conclusion

Content uniformity is considered a critical quality attribute for low-dose solid dosage forms. Direct compression is one of the simplest methods of tablet production, which is feasible in low-dosage formulations, where the mechanical properties of the dry powder blend (dry mixture) primarily derive from the properties of the excipients. The active substance content uniformity depends on the efficiency of the mixing process to obtain a uniform dry mixture. Therefore, it is of primary importance to optimize the dry mixing process in order to achieve appropriate content uniformity of the active substance in the dry mixture from which the tablets are produced.

In our study, we have employed DoE for the optimization of dry mixing of low-dose Alprazolam powder blends. Two ingredient DoE models were used to assess the effects of process variables and filler type (morphology, particle size and particle size distribution) on the content uniformity of the powder blends. Considering the large number of experiments and samples to be analyzed, we've used a validated Raman spectroscopy technique for the rapid determination of Alprazolam content in powder blends.

Taking into account the poor performance of the "in bulk" cube mixer, only the high-shear dry mixing process using Tablettose 80 as filler was optimized. The most optimal conditions were achieved using a mixing time of 2 minutes, a mixing speed of 300 rpm and a 70% filling level of the mixer. These process conditions were used to produce three independent lab batches of low dose Alprazolam tablets which conformed to the requirements of the European Pharmacopoeia for Alprazolam content and uniformity of the dosage units.

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Pristup baziran na Dizajnu eksperimenata za poboljšanje procesa suvog mešanja u proizvodnji tableta sa niskom dozom Alprazolama uz primenu Raman spektroskopije za praćenje uniformnosti sadržaja

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Kratak sadržaj

Razvijena je formulacija tableta sa niskom dozom Alprazolama, potentnog benzodiazepinskog derivata, imajući u vidu da je postizanje odgovarajuće uniformnosti sadržaja aktivne supstance u praškastim mešavinama i tabletama veliki izazov. Upotrebljene su dve vrste laktoze monohidrata (Tablettose 80 i Granulac 200) i dva tipa procesa suvog mešanja *(high-shear* i *"in bulk"*). Da bi se procenio uticaj varijabli (brzina mešanja, vreme mešanja, nivo punjenja *high-shear* i kockastog miksera, vrste laktoze monohidrata) i njihove interakcije na odgovor (uniformnost sadržaja Alprazolama u praškastim mešavinama), generisan je *Factorial* 2^4 dizajn (4 faktora, 2 nivoa, 1 blok) za svaku vrstu miksera. Za *high-shear* mešanje korišćen je *Response Surface, D-optimal Factorial* 2^4 dizajn (2 replikacije i 31 eksperiment), dok je za *"in bulk"* mešanje korišćen *Response Surface, Central Composite Factorial* 2^4 dizajn (34 eksperimenata). Procesni parametri *high-shear* miksera su varirani u sledećim opsezima: nivo punjenja od 70-100%, brzina impelera od 50-300 rpm i vreme mešanja od 2-10 minuta. Za kockasti mikser upotrebljeni su sledeći opsezi procesnih parametra: nivo punjenja od 30-60%, brzina mešanja od 20-390 rpm i vreme mešanja od 2-10 minuta. *Raman* spektroskopija zajedno sa validiranim modelom *Partial Least Square (PLS)* regresije je korišćena kao *Process Analytical Technology (PAT)* alatka za određivanje sadržaja i praćenje uniformnosti sadržaja Alprazolama. *Design of Experiments, (DoE)* model je primenjen za optimizaciju procesa mešanja u pogledu postizanja

odgovarajuće uniformnosti sadržaja Alprazolama koristeći *high-shear* mešanje i filler Tablettose 80. *Desirability function* otkriva da bi primena sledećih procesnih parametara: vreme mešanja od 2 minuta, brzina mešanja od 300 rpm i nivo punjenja miksera od 70%, omogućila proizvodnju praškastih mešavina sa najnižom varijabilnošću sadržaja Alprazolama. Tri nezavisne laboratorijske serije tableta sa niskom dozom Alprazolama, proizvedene *high-shear* mešanjem sa ovim procesnim parametrima, odgovaraju zahtevima Evropske farmakopeje za sadržaj Alprazolama i uniformnost doziranih jedinica.

Ključne reči: Alprazolam, uniformnost sadržaja, laktoza monohidrat, *Response Surface Factorial* eksperimentalni dizajn, *Raman* spektroskopija

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