

Influence of process parameters on content uniformity of a low dose active pharmaceutical ingredient in a tablet formulation according to GMP

JAN MUŠELÍK¹
ALEŠ FRANČ¹
PETR DOLEŽEL¹
ROMAN GONĚC²
ANNA KRONDLOVÁ¹
IVANA LUKÁŠOVÁ¹

¹ Department of Pharmaceutics
Faculty of Pharmacy
University of Veterinary
and Pharmaceutical Sciences
612 42 Brno, Czech Republic

² Masaryk Memorial Cancer Institute
61242 Brno, Czech Republic

The article describes the development and production of tablets using direct compression of powder mixtures. The aim was to describe the impact of filler particle size and the time of lubricant addition during mixing on content uniformity according to the Good Manufacturing Practice (GMP) process validation requirements. Processes are regulated by complex directives, forcing the producers to validate, using sophisticated methods, the content uniformity of intermediates as well as final products. Cutting down of production time and material, shortening of analyses, and fast and reliable statistic evaluation of results can reduce the final price without affecting product quality. The manufacturing process of directly compressed tablets containing the low dose active pharmaceutical ingredient (API) warfarin, with content uniformity passing validation criteria, is used as a model example. Statistic methods have proved that the manufacturing process is reproducible. Methods suitable for elucidation of various properties of the final blend, *e.g.*, measurement of electrostatic charge by Faraday pail and evaluation of mutual influences of researched variables by partial least square (PLS) regression, were used. Using these methods, it was proved that the filler with higher particle size increased the content uniformity of both blends and the ensuing tablets. Addition of the lubricant, magnesium stearate, during the blending process improved the content uniformity of blends containing the filler with larger particles. This seems to be caused by reduced sampling error due to the suppression of electrostatic charge.

Keywords: content uniformity, warfarin, validation, narrow therapeutic index, Faraday pail, PLS regression

Accepted April 2, 2014

Development of dosage forms demands pharmacological and technological aspects, including suitably chosen statistic provability, to be taken into account from the very beginning. In narrow therapeutic index drugs, content uniformity is a critical parameter.

* Correspondence; e-mail: franca@vfu.cz

Strict Good Manufacturing Practice (GMP) requirements concerning process validation of content uniformity to be met when transferring the production to the industrial scale should be followed as early as during laboratory development.

Pharmacological aspects of tablet blend content uniformity

Warfarin is used either as sodium salt or sodium salt isopropanol clathrate. Developed initially as rat poison, warfarin is still used as a first-line drug for thrombosis prevention in the USA, where tablets containing warfarin sodium salt have been marketed as Coumadine by DuPont Pharma since the 1950s. Patent protection expired by 1962; however, the narrow therapeutic index of warfarin prevented the production of generic substitutes. In 1980, switch to generic warfarin in the Boston City Hospital led to increased risk of mortality and health complications including acute bleeding (1). Wittkowsky suggests that extensive content uniformity limits may cause such complications. For example, a generic warfarin tablet labeled as containing 10 mg of drug might contain 8.50–11.50 mg according to EP 2.9.6 specification (85.0–115.0 %). The impact of this overlap is significant considering that when warfarin dosage is adjusted in response to alterations in therapy intensity, the dosage is typically increased or decreased by only 5–15 % of the daily dose. The original producer DuPont Pharma used the internal limit for content uniformity of 92.5–107.5 % with relative standard deviation (RSD) no more than 3 %, while the generic producer met the United States Pharmacopoeia (USP) limits of 85–115 % with RSD no more than 6 % (2). On the other hand, Jaffer and Bragg argued that the generic product contained amorphous warfarin, whereas the original product contained the salt in crystalline form. Amorphous warfarin could not be used in warfarin tablets in the USA any more (3). Until 1996, DuPont remained the sole producer of warfarin tablets in the USA. In September 1997, Food and Drug Administration (FDA) approved generic warfarin containing warfarin sodium salt clathrate, marketed by Barr Pharmaceuticals (now Teva). DuPont tried to stop the oncoming generic product and in 1996 asked the FDA to establish stricter limits in bioequivalence testing and to adopt its internal content uniformity limit as the USP standard. FDA declined both suggestions (4). Studies by Halkin (2003) (5) and Swenson (2005) (6) proved that the existing bioequivalence testing of generic substitutes was sufficient. Nevertheless, two important generic producers of warfarin tablets – Taro Pharmaceuticals and Apotex – introduced stricter internal limits for both bioequivalence and content uniformity, *i.e.*, AUC and c_{max} within 0.8–1.25 with a confidence level 0.95 instead of 0.90, content uniformity within 92.5–107.5 % of the average and RSD not more than 3 % (7). In 2006, Barr Pharmaceuticals acquired the Croatian pharmaceutical producer Pliva Zagreb, together with its patent for warfarin tablets marketed in the USA. Content uniformity of these tablets was high, the content in all strengths ranged within 97–103 % of the average and RSD was not more than 2 %. The patent granted to the Croatian producer Pliva described warfarin tablets with possibly the highest content uniformity achieved so far (8). The procedure was based on the carrier impregnated with aqueous solution of warfarin sodium salt clathrate. Regardless of being crystalline or amorphous, the structure of warfarin used did not change after drying and further processing (9). It is clear that content uniformity remains an important issue when formulating solid dosage forms of drugs with a narrow therapeutic index. Similarly, Benet claims that complications during the treatment with narrow therapeutic index drugs API are mainly caused by inter-individual variability in drug quality (10).

Current GMP legislation concerning content uniformity

Regardless of how strict are the corporate internal limits for content uniformity, the quality of the product has to be tested in regular production according to the GMP. In fact, current legislation was inspired by Barr Laboratories, because in early 1990s FDA investigation in this company detected problems in content uniformity testing of solid dosage forms (11). In 1993, this resulted in an arbitrary court decision, ordering content uniformity testing of blends irrespective of validation process adequacy (12). Before that time, USP required content uniformity of 10 samples to fall within 85–115 % of the average and RSD not more than 6 %; this was narrowed to 90–110 % and RSD not more than 5 %. Sample mass should not exceed more than three times that of the final dosage form (12). These limits were listed in the FDA guidance (13). The decision was criticized, since there was no suitable sampling method that would eliminate sampling error. Parenteral Drug Association (PDA) reacted by issuing its Technical Report No. 25, suggesting a »holistic approach« to general analysis of the product. Under defined conditions, the error of failed content uniformity of the blend could be reconstructed from the error of passed content uniformity of the final product. However, the average content of the blend and the final product have to be statistically similar. More samples are required, both from one sampling place and from several sampling places (sample stratification). The error of blend content uniformity is equal to the sum of errors within one sampling place and product error within several sampling places (12). Following the discussion between the Product Quality Research Institute (PQRI), PDA and FDA, the latter issued a draft of new guidance (14), including a commentary (15), containing a more extensive description of the original limits based on stratified sampling. The FDA guidance documents were issued as the final guidance for industry and represent FDA's current thinking on the topics covered. However, FDA's guidance documents are not compulsory for either industry or the FDA. FDA accepts alternative approaches to those described in guidance documents, as long as the chosen approach is in accord with the applicable statute or regulation. On the other hand, USP monographs, harmonized with European Pharmacopoeia (EP) monographs, are obligatory.

Content uniformity validation

In the course of validation, the product has to meet defined requirements and specifications at a statistically significant confidence level. Validation covers also the stage of development, *i.e.*, selection of excipients, procedure, and process parameters, including manufacturing process control, intermediates, final product and sampling. Content uniformity is a key parameter (16). Statistic methods offer several sophisticated models to evaluate the significance of the process (*e.g.*, ANOVA). Nevertheless, experience suggests that complex equations demand professional statisticians. There are several simpler models where values are checked against tabulated data or are calculated easily. Use of capability indices K , C_p and C_{pk} enables the personnel to assess the correctness and precision of the process based on simple parameters demanded by the pharmacopoeia: average, maximum, minimum, and standard deviation (17). C_{pk} index is defined as the lower value of upper and lower indices.

$$\widehat{C_{pk}} = \min \left[\frac{USL - \bar{x}_i}{3s}, \frac{\bar{x}_i - LSL}{3s} \right] \quad i(1)$$

USL – upper specified limit, LSL – lower specified limit, $\bar{x}_i$ – average, s – standard deviation.

If the result is not less than 1.0, at least 99.73 % of batches produced by this process will pass the applied acceptability criterion. This limit is suitable for content uniformity validation. Other simple methods include standard deviation predilection interval (SDPI) or Bergum division adapted to suit the USP monograph »uniformity of dosage units« <905> and its harmonized European counterpart (2.9.40). Bergum criterion is based on tabulated RSD value which guarantees, with 90.0 % certainty, that at least 95.0 % of samples tested for content uniformity will pass the <905> USP test (18).

Impact of composition and procedure on content uniformity

To reach uniform content of a low dose drug in the final dosage form while keeping the production costs low, a suitable tablet composition and procedure have to be chosen. The easiest method for tablet production is direct compression, where all constituents are weighed and mixed in a single container. Turbula is a reliable and efficient 3D mixing device that can be used not only in the development but also in scale-up production (19). With this device, final content uniformity is influenced by the ratio of constituents and the active ingredient, electrostatic charge, particle density, shape and size. Significant procedural parameters include the mixing order of individual components, mixing time and speed, container shape and container load (20). The highest degree of content uniformity can be reached if the shape, size and density of mixed particles are as similar as possible; spherical shape is preferred. The ratio of active substance and excipients should be equal and there should not be any electrostatic charge (21). If the mixing time is too short, the blend is not mixed properly; on the other hand, if the mixing time is too long, »overmixing« can occur and the constituents will separate in dependence on their differing properties. The results are almost impossible to estimate in advance and the above mentioned recommendations cannot be fulfilled. Particle size, shape and density are usually dissimilar and electrostatic charge changes in the course of the manufacturing process (22). Applied composition is thus a compromise between general recommendations and real requirements.

Experiment design and hypothesis

The goal was to assess the impact of particle size distribution of the filler, in this case calcium hydrogen phosphate, on content uniformity of the blend in relation to the addition of magnesium stearate at various stages of the mixing process. The composition is based on previous experiments (23); the procedure of »common blend« was used where tablets of various strengths (1 to 10 mg) were compressed from the same blend by changing the tablet mass (approximately 50 to 500 mg). A single validated blend containing 2 % of warfarin sodium salt clathrate was used for the production of tablets of all strengths.

Content uniformity of the tableting blend had to meet EP (2.9.6) and FDA requirements and the results were evaluated using the *C_{pk}* index. Final product content uniformity had to meet USP (<905>) and EP (2.9.6, 2.9.40) requirements and the results were evaluated using the *C_{pk}* index and Bergum division. Tablets had to meet also internal DuPont requirements of 92.5–107.5 % of the labeled drug strength, and RSD not more than 3 %.

We planned to find out if demixing of the blend during the process could be caused by electrostatic charge forming in the blend during blending.

Partial least squares (PLS) regression was used to fully evaluate the impact of studied variables on content uniformity.

EXPERIMENTAL

Tableting blend and tablets

Blend composition and some of its physicochemical properties are listed in Table I. All constituents were sieved through a 250 μm sieve and mixed for 15 minutes (procedure A) or all constituents, without magnesium stearate, were mixed for 10 minutes, then magnesium stearate was added and another 5 minutes of mixing followed (procedure B). A Turbula homogenizer (T2C, Switzerland) was used, speed 40 rpm. Mass of one batch was 500.0 g. Four blends were prepared (2 compositions, 2 procedures). Flat tablets with a diameter of 7 mm, mass about 200 mg, and hardness about 70 N were produced using an eccentric press (Korsch EK0, Germany). Two batches were produced for every procedure/composition combination. An additional third batch was produced using the composition and procedure that showed the best results.

Sampling and warfarin content measurement

Tableting blend was placed in a cylindrical vessel of 25 cm diameter and was leveled to a height of about 2 cm by slight horizontal movement. The area was then divided evenly into 10 parts; out of each of these parts, a sample weighing approximately three times more than the tablet was taken with a small spoon. Samples were weighed with 0.1 mg

Table I. Composition of the blend and physical properties of individual constituents

Component	Producer	Density [kg m^{-3}]	Particle size (μm) ^a			Content (%)	
			D_{10}	D_{50}	D_{90}	I	II
Warfarin	Pliva (Croatia)	1312.8	2.5	10.3	72.4	2.0	2.0
Di-cafos 92-12	Budenheim KG (Germany)	2881.5	36.1	61.1	96.0	70.0	–
Di-cafos 92-14	Budenheim KG (Germany)	2937.9	2.4	152.3	309.8	–	70.0
Avicel pH 101	FMC BioPolymer (USA)	1572.4	14.7	46.3	110.7	25.0	25.0
Ac-Di-Sol	FMC BioPolymer (USA)	1611.5	12.2	33.1	86.7	2.0	2.0
Magnesium stearate	Peter Greven (Germany)	1085.9	2.6	10.2	23.1	1.0	1.0

^a D_x = x % of measured particles smaller than this size (μm); Di-cafos – calcium hydrogen phosphate; Avicel PH 101 – microcrystalline cellulose; Ac-Di-Sol – sodium croscarmellose

precision, transferred quantitatively to 100 mL flasks and water was added. Tablets were sampled during the tableting process so that 10 tablets from each batch were taken at regular intervals. Tablets were weighed, put into flasks and let to dissolve for 12 hours in a mixture of water and methanol (9:1, V/V). From both the blend and tablet samples, insoluble excipients were removed by a centrifuge for 10 minutes and at 15000 rpm (SIGMA Laborzentrifugen 2K15, Germany) and samples were analyzed by HPLC. Warfarin content was measured using the calibration curve.

We used a high performance liquid chromatograph YL 9100 (Young Lin Instrument, Korea) with a quaternary pump, automatic sampler and diode array detector. The separation column was BDS HYPERSIL C18 (150 × 4.6 mm; particle size 5 µm). Analysis conditions: mobile phase methanol (64 %) and formic acid (0.04 mol L⁻¹, 36 %), flow rate 1.4 mL min⁻¹, column temperature 25 °C, analysis wavelength 280 nm, sample size 20 µL, analysis time 7 minutes.

Charge measurement

The charge was measured in individually prepared model mixtures. Their composition was designed so as to find out the impact of the carrier particle size along with addition of magnesium stearate on the final electrostatic charge. For the preparation of model mixtures, an identical procedure was applied as in the preparation of tableting blends. Fillers alone (Di-cafos 92–12, Di-cafos 92–14), their mixtures with warfarin and their mixtures with warfarin and magnesium stearate were used as model samples. Fillers alone and their mixtures with warfarin were mixed for 10 minutes; when magnesium stearate was added, additional 5 minutes of mixing followed.

Electrostatic charge was measured using a Faraday pail of standard construction, consisting of the outer insulating and inner measurement pail (24). The sample transfers its charge to the inner pail and voltage change between the inner pail and the ground is measured. Complete volume of the model mixture weighing 80 g (measurement repeated three times) was poured directly from the glass homogenization vessel to the inner cup of 250 mL volume and the measured charge was calculated to correspond to 1 g of sample.

Statistical data analysis

Results of individual samples ($n = 10$) from each batch were recalculated according to the theoretical warfarin content in the blend. Average content, standard deviation and relative standard deviation were calculated for every batch. Statistical evaluation of variance for both compositions and both procedures was performed using the *F*-test of equality of variances (QC.Expert 3.2., TriloByte). To evaluate the impact of procedure variables (filler particle size, time of magnesium stearate addition) and their interaction with response variables (RSD, *Cpk* of EP 2.9.6 criteria, *Cpk* of FDA criteria), PLS regression was used, including the Martens uncertainty test (25). Prior to modeling, response variables were automatically adjusted by autoscaling, which uses mean-centering followed by the dividing of each variable by its standard deviation. Design evaluation was performed with Unscrambler X (v. 10.3, Camo software).

RESULTS AND DISCUSSION

Blends and tablets that were manufactured differed in the filler particle size distribution and in the time when lubricant was added in the mixing process. Mixing time of 15 minutes was chosen on the basis of previous experiments with similar composition where the impact of mixing time, different types of fillers and different particle size distribution of warfarin on content uniformity were tested (23, 26). Magnesium stearate was added either at the beginning of mixing or after 10 minutes. The results show that the chosen variables affected warfarin content uniformity of the tableting blend (Table II). If the filler with larger particle size ($D_{50} = 152 \mu\text{m}$) was used, content uniformity was better than in the case of smaller particle size ($D_{50} = 61 \mu\text{m}$). Results for both procedures and compositions were compared by the F-test of equality of variances ($p \leq 0.05$). Statistical evaluation proved a significantly smaller variance of the measured content in blends prepared with fillers of larger particle size (Di-cafos 92-14; $D_{50} = 152 \mu\text{m}$). This applies to both procedures, which differed in the time when lubricant was added. The influence of the procedure used on the final variance of warfarin content was also evaluated by the F-test of equality of variances. The test did not reveal any statistically significant difference in the variance of warfarin content in blends prepared by procedures A and B (Table II). This applied to blends with both larger and smaller filler particle size. This points to the conclusion that when using either procedure, the critical parameter for reaching the required content uniformity is the filler particle size distribution.

The time when lubricant was added did not have a statistically significant impact on the measured variance of warfarin content in manufactured blends. Physical bonds between magnesium stearate and lubricated particles were weaker than in the absence of lubricant (27). Magnesium stearate may influence the intensity of physical bonds, *i.e.*, elec-

Table II. Average content and content uniformity parameters of blends

Batch ^a	$\bar{x}^b$ (%)	RSD	EP ^c (2.9.6)	FDA ^c	Cpk^d (2.9.6)	Cpk^d (FDA)
1_A_D61	103.0	6.24	+	–	0.62	0.36
2_A_D61	100.3	5.53	+	–	0.88	0.58
1_A_D152	98.0	3.06	+	+	1.45	0.89
2_A_D152	100.1	2.58	+	+	1.92	1.27
1_B_D61	103.3	3.41	+	+	1.07	0.62
2_B_D61	102.3	7.18	–	–	0.56	0.34
1_B_D152	100.5	1.37	+	+	3.50	2.29
2_B_D152	99.3	1.76	+	+	2.72	1.77
3_B_D152	102.2	2.06	+	+	2.04	1.24

^a Batch label: batch number_procedure_filler particle size D_{50} ; (50 % of particles smaller either than 61 or 152 μm);

^b average of 10 samples from a particular batch; ^c (+) passed, (–) failed; ^d Cpk values calculated according to Equation 1.

Table III. Total electrostatic charges of model blends

Components ^a	Electrostatic charge (nC g ⁻¹) ^d
D61 ^a	0.042 ± 0.031
D61 + W ^b	0.083 ± 0.014
Blend with D61 ^c	-0.165 ± 0.041
D152 ^a	0.042 ± 0.007
D152 + W ^b	0.083 ± 0.019
Blend with D152 ^c	-0.054 ± 0.007

^a Di-cafos only (D61 or D152 according to particle size); ^b blend of Di-Cafos with warfarin (W); ^c tableting blend prepared by procedure B (*i.e.*, magnesium stearate was added after 10 minutes of blending); ^d average of three measurements ± standard deviation.

trostatic bonds and Van der Waals bonds, which can have an impact on content uniformity by decreasing the electrostatic charge of blended part.

Electrostatic charge was measured to try to explain the observed impact of filler particle size on content uniformity. The impact of added magnesium stearate on the magnitude of electrostatic charge was measured with the Faraday pail in model blends containing the active substance only (warfarin sodium salt clathrate) and filler (calcium hydrogen phosphate) of various particle sizes (Table III). The composition of these model mixtures was chosen so as to eliminate the impact of other excipients (Avicel and Ac-Di-Sol) on the final electrostatic charge.

Measurements showed that neither filler itself nor its blends with warfarin differed in charge in a statistically significant way. However, when magnesium stearate was added, statistically significant changes in electrostatic charge occurred. When the filler of larger particle size was used, the change in electrostatic charge was lower than in the filler of smaller particle size. When magnesium stearate was added, the value of electric charge changed its polarity. This influence of magnesium stearate was already described (27). These results may explain better uniformity of blends containing the filler of larger particle size because the electrostatic charge was reduced. Charge can have a negative impact on uniformity or increase the possibility of sampling error. Experience shows that if the blend has lower content uniformity than tablets and there is no further homogenization during compression, there has to be an error in blend sampling (12). As the difference in uniformity of individual blends was higher than in uniformity of tablets produced from them (see Table II and V), this was probably a sampling error (*e.g.*, adhesion of blend components to the sampler surface caused by electrostatic charge). Particle size of mixed constituents has an impact on uniformity by itself (*e.g.*, movement of particles) or in connection with some other commonly used excipients, *e.g.*, magnesium stearate, which influences physical bonds between particles with respect to their size.

Although experimentation was run on a laboratory scale, tableting blends were evaluated according to validation criteria used in production transfer on a larger scale, corresponding to GMP requirements for validation. We used criteria from EP 2.9.6, which states

Table IV. PLS-ANOVA summary table. *P*-values of the effects of particular procedural variables, and their interactions with response variables. Values with $p < 0.1$ are in bold

	RSD	Cpk (EP 2.9.6)	Cpk (FDA)
A	0.322	0.355	0.358
B	0.322	0.355	0.358
D61	0.005	0.011	0.010
D152	0.005	0.011	0.010
A*D61	0.084	0.076	0.079
A*D152	0.702	0.636	0.635
B*D61	0.259	0.307	0.302
B*D152	0.031	0.096	0.094

Table V. Average content and content uniformity parameters of tablets

Batch ^a	$\bar{x}_i^b$ (%)	RSD	EP ^c (2.9.6)	EP ^c (2.9.40)	Cpk ^d (2.9.6)	DuPont ^c specification	Bergum division ^c
1_A_D61	101.4	3.57	+	+	1.26	–	–
2_A_D61	102.0	5.06	+	+	0.84	–	–
1_A_D152	101.7	2.58	+	+	1.67	+	+
2_A_D152	101.0	2.62	+	+	1.77	+	+
1_B_D61	100.1	3.20	+	+	1.55	–	–
2_B_D61	103.2	1.62	+	+	2.36	+	+
1_B_D152	102.3	2.52	+	+	1.64	+	+
2_B_D152	99.9	1.67	+	+	2.96	+	+
3_B_D152	102.2	2.54	+	+	1.65	+	+

^a Batch label: batch number_procedure_filler particle size D_{50} ; (50 % of particles smaller either than 61 μm or 152 μm); ^b average of 10 samples from a particular batch; ^c (+) passed, (–) failed; ^d Cpk values calculated according to Equation 1.

that the content of active substance in each of 10 samples has to be within 85–115 % of average content as well as criteria from FDA guidance which states that each sample has to contain 90–110 % of average content and RSD has to be lower than 5 %. Cpk indices were calculated for both EP and FDA limits: for validation of solid dosage forms, Cpk must not be less than 1.0. Tableting blends prepared from the filler of larger particle size met all validation criteria, which is consistent with the above mentioned facts. The time when magnesium stearate was added to the blend was found to be significant; only batches where magnesium stearate was added in the course of mixing met the FDA requirement,

confirmed at the required statistical significance level $Cpk \geq 1$ (Table II). Although the current FDA guidance no longer considers the use of traditional three-batch validation appropriate, it does not prescribe the number of validation batches for a prospective validation protocol (28). A third batch based on this procedure and composition was prepared and expected to strengthen statistical data and help scale up the production. This third batch also met all validation criteria. PLS regression was used to fully describe the impact of studied technological variables (filler particle size, time of lubricant addition) on response variables (RSD, Cpk pursuant to EP 2.9.6 criteria, Cpk pursuant to FDA criteria) of prepared blends. One of the PLS model outputs is the PLS-ANOVA summary table, which shows p -values of the effects of respective procedural variables and their interactions with response variables. If the p -value of effect is not higher than 5 %, the effect is considered to be significant. If the value ranges between 5 and 10 %, the effect is marginally significant. Design evaluation by PLS regression is suitable because there is no limit to the number of experiments. Co-variance between response variables is taken into account, which enables a study of their mutual dependence. Graphic simplification of dependence between procedural and response variables is usually presented as a correlation loading plot, where significant correlations ($R^2 > 0.5$) are marked by Hotelling's ellipse. An advantage is the possibility of using un-controlled response variables in the experiment and finding out if they have an impact on the quality of the model. On the other hand, it must be said that it is not possible to get real p -values but only their estimation (29, 30).

The developed PLS model describes quantitatively the dependence between the matrix of X procedural variables (filler particle size, time lubricant addition) and the matrix

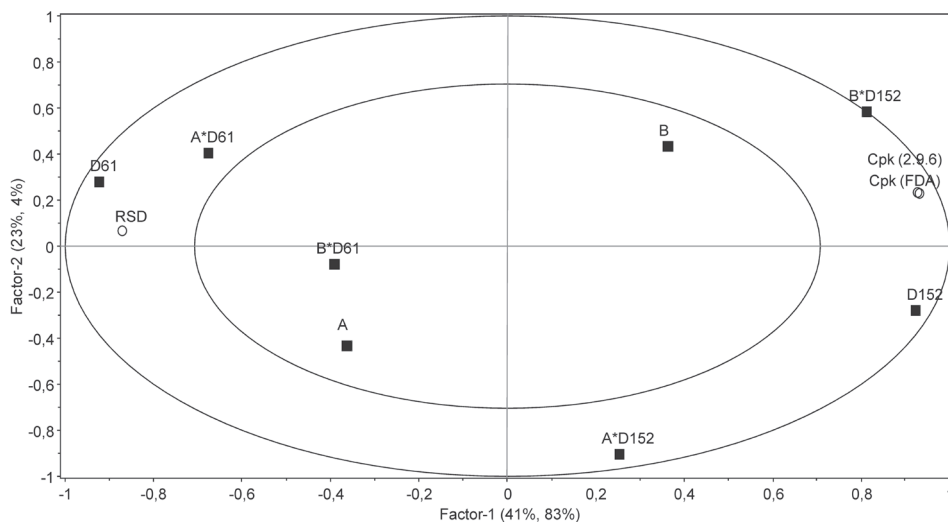


Fig. 1. Correlation-loading plot showing relationships between the procedural variables and their interactions of X -matrix (boxes) and responses of Y -matrix (circles). Technological variables labeled in the form of the applied procedure (A or B), filler particle size (D61 or D152) and their interactions (A*D61; A*D152; B*D61; B*D152). Response variables labeled in the form of RSD, Cpk for EP 2.9.6 criteria and Cpk for FDA criteria.

of Y response variables (RSD, *Cpk* pursuant to EP 2.9.6 criteria, *Cpk* pursuant to FDA criteria). The resulting correlation loading plot (Fig. 1) describes, using the first two components, 63 and 87 % of total variability of matrices X and Y, respectively. The distribution of variables in the outer area of Hotelling's ellipse suggests a significant impact ($p < 0.05$) of particle size (D61; D152) on all response variables, which is quantitatively shown in Table IV. The results also show a significant impact ($p < 0.05$) of interaction procedure B *vs.* larger particles (B*D152) on RSD value. It is possible to claim that the use of procedure B in combination with larger particles caused a significant decrease in RSD and a potentially significant increase ($0.05 \leq p < 0.1$) in both *Cpk* values. This is the reason why this combination of procedure B and larger filler particle size (B*D152) can be considered more suitable for attaining good content uniformity than the combination of procedure A and smaller filler particle size (A*D61). There is a potentially significant impact ($0.05 \leq p < 0.1$) on increased RSD and decreased *Cpk*. Figure 1 shows a narrow correlation between both *Cpk* values, which is also clear from the minimum difference between *p*-values in Table I. These findings confirm the uniformity results according to validation criteria, when all criteria were met only by blends prepared by procedure B from the filler with larger particle size.

Blends were processed into tablets, in which warfarin content was measured, evaluated statistically and according to validation criteria similarly to the tableting blend (Table V). The results obtained from tablets originating from fillers of different particle size (procedure A or B) were compared by the *F*-test of equality of variances ($p \leq 0.05$). For procedure A, this statistical evaluation proved a significantly lower variance of the measured content of active substance in tablets when the filler of larger particle size was used. For procedure B, no statistically significant impact was found. As there was a statistically significant difference between tableting blends and tablets from the same batches, it seems probable that content uniformity was correct but there was a sampling error. This claim corresponds to the measured growth of overall electrostatic charge in blends prepared from the filler of smaller particle size (Table III). Electrostatic charge can have a negative impact on sampling, which was probably the main cause of poorer content uniformity of these blends. Similarly to the blend, there was no statistically significant difference in the measured contents in tablets (*F*-test of equality of variances) between procedures A and B, *i.e.*, the time when lubricant was added to the blend. Tablets were evaluated according to validation criteria (Table V) common for the production of solid dosage forms. EP monographs 2.9.6 and 2.9.40, *Cpk* index for 2.9.6 monograph limits (85–115 %) and Bergum division were used. Bergum division offers tabulated RSD values that ensure, on a 90 % confidence level with 95 % probability, that subsequently produced batches will pass EP monograph 2.9.40. Validation criteria were met only in tablets manufactured with the filler of larger particle size.

CONCLUSIONS

The paper describes the methods used in process validation of solid dosage form manufacture. A model dosage form, tablets with low API content, containing warfarin, was used to evaluate the impact of particle size distribution of the used filler and the time when lubricant was added on blend and tablet content uniformity. Content uniformity was evaluated according to validation and pharmacopoeial criteria. The results show that both evaluated variables (filler particle size and time of lubricant addition) had an impact on

content uniformity. The highest content uniformity of blend and tablets was found for the filler of particle size $D_{50} = 152 \mu\text{m}$ combined with magnesium stearate added later in the blending process (technology B). *Cpk* index and Bergum division proved that this blend and tablets meet pharmacopoeial criteria as well as FDA GMP scale-up content uniformity criteria at a statistically high level. Differences between blends and tablets manufactured from them ($D_{50} = 61 \mu\text{m}$, technology B) are probably caused by the blend sampling error. Electrostatic charge formed during the blending may play a major role in this error. Electrostatic charge measurements of model mixtures are in accord with the assumption that a higher electrostatic charge of the mixture may cause a blend sampling error (e.g., adherence of material to sampler surface). This experience should contribute to the future development of safe drugs containing a small amount of highly efficient APIs manufactured by direct compression.

Abbreviations. – API – active pharmaceutical ingredient, EP – European Pharmacopoeia, FDA – Food and Drug Administration, GMP – good manufacturing practice, NTI – narrow therapeutics index, PDA – parenteral drug association, PLS – partial least square, PQRI – product quality research Institute, RSD – relative standard deviation, SDPI – standard deviation predilection interval, USP – United States Pharmacopoeia, WHO – World Health Organization.

Acknowledgements. – This work was supported by Project IGA VFU Brno 39/2011/FaF.

REFERENCES

1. L. M. Vercaigni and G. G. Zhanel, Clinical significance of bioequivalence and interchangeability of narrow therapeutic range drugs: Focus on warfarins, *J. Pharm. PharmSci.* **1** (1998) 92–94.
2. A. K. Wittkowsky, Generic warfarin: Implications for patient care, *Pharmacotherapy* **17** (1997) 640–643; DOI: 10.1002/j.1875-9114.1997.tb03741.x.
3. A. Jaffer and L. Bragg, Practical tips for Warfarin dosing and monitoring, *Clev. Clin. J. Med.* **70** (2003) 361–371; DOI: 10.3949/ccjm.70.4.361.
4. United States Court of Appeals, Third Circuit. Court decision in re: Warfarin sodium antitrust litigation, 391 F.3d 516, December 2004; <https://bulk.resource.org/courts.gov/c/F3/391/391.F3d.516.02-3758.02-3757.02-3755.02-3603.html>; access date October 15, 2013.
5. H. Halkin, A. Shapiro, D. Kurnik, R. Loebstein, V. Shalev and E. Kokia, Increased warfarin doses and decreased international normalized ratio response after nationwide generic switching, *Clin. Pharmacol. Ther.* **74** (2003) 215–221; DOI: 10.1016/S0009-9236(03)00166-8.
6. C. N. Swenson and G. Fundak, Observational cohort study of switching warfarin sodium products in a managed care organization, *Am. J. Health-Syst. Pharm.* **57** (2000) 452–455.
7. A. E. Sawoniak, A. F. Shalansky, P. J. Zed and R. Sundreji, Formulary considerations related to warfarin interchangeability, *Can. J. Hosp. Pharm.* **55** (2002) 215–218.
8. A. Franc, B. Žaludek, R. Goněč, M. Maleček, H. Tkadlečková and A. Petrovičová, *Method of Producing Dosage Units of a Solid Drug Form Containing Warfarin Sodium Salts as Active Component*, WO Pat. 2005034919 17 October 2003.
9. A. Franc, M. Rabišková and R. Goněč, Impregnation: a progressive method in the production of solid dosage forms with low content of poorly soluble drugs, *Eur. J. Parent Pharm. Sci.* **16** (2011) 85–93.
10. L. Z. Benet and J. E. Goyan, Bioequivalence and narrow therapeutic index drug, *Pharmacotherapy* **15** (1995) 433–440; DOI: 10.1002/j.1875-9114.1995.tb04379.x.
11. *The »Barr Laboratories« Court decision*, United States District Court for the District of New Jersey, Civil action No. 92-1744, January 2004; <http://www.navigategmp.com/pdf/BarrLabs.pdf>; access date October 19, 2013.

12. J. Berman, D. E. Elinski, C. R. Gonzales, J. D. Hofer, P. J. Jimenez, J. A. Planchard, R. J. Tlachac and P. F. Vogel, Blend uniformity analysis: Validation and in-process testing. Technical Report No. 25. PDA (Parenteral Drug Association), *PDA J. Pharm. Sci. Technol.* **51** (1997) S1-99.
13. FDA Guidance for industry, *ANDAs: Blend Uniformity Analysis*, Draft guidance, August 1999; <http://www.fda.gov/OHRMS/DOCKETS/98fr/992635gd.pdf>; access date October 19, 2013.
14. FDA Guidance for industry, *Powder Blends and Finished Dosage Units – Stratified In-process Dosage Unit Sampling and Assessment*, Draft guidance, October 2003; <http://www.fda.gov/OHRMS/DOCKETS/98fr/03d-0493-gdl0001.pdf>; access date October 19, 2013.
15. FDA Guidance for industry, *Powder Blends and Finished Dosage Units – In-process Blend and Dosage Unit Inspection (Sampling and Evaluation) for Content Uniformity*, Revised draft guidance, January 2004; http://www.fda.gov/ohrms/dockets/dailys/04/jan04/013004/03D-0493_emc-000003-01.pdf; access date October 19, 2013.
16. FDA reference material, *Oral Solid Dosage Forms Pre/post Approval Issues (1/94)*, Guide to inspections of oral solid dosage forms, pre/post approval issues for development and validation, January 1994. <http://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm074928.htm>; access date 19 October, 2013.
17. P. Cholleyudth, Establishing acceptance limits for probability of passing multiple stage tests in process validation through a process capability approach, *J. Valid. Tech.* **15** (2009) 77–90.
18. J. S. Bergum and H. Li, Acceptance limits for the new ICH USP 29 content-uniformity test, *Pharm. Tech.* **31** (2007) 90–100.
19. J. Kushner, Incorporating Turbula mixers into a blending scale-up model for evaluating the effect of magnesium stearate on tablet tensile strength and bulk specific volume, *Int. J. Pharm.* **429** (2012) 1–11; DOI: 10.1016/j.ijpharm.2012.02.040.
20. J. Zheng, *Formulation and Analytical Development for Low-Dose Oral Drug Products*, John Wiley & Sons, New Jersey 2009, pp. 169–196.
21. J. Hilden, M. Schrad, J. Kuehne-Willmore and J. Sloan, A first-principles model for prediction of product dose uniformity based on drug substance particle size distribution, *J. Pharm. Sci.* **101** (2012) 2364–2371; DOI: 10.1002/jps.23130.
22. Y. Pu, M. Mazumder and C. Cooney, Effects of electrostatic charging on pharmaceutical powder blending homogeneity, *J. Pharm. Sci.* **98** (2009) 2412–2421; DOI: 10.1002/jps.21595.
23. J. Muselík and A. Franc, Evaluation of content uniformity of tablets with a low content of the active ingredient with a narrow therapeutic index, *Ces. Slov. Farm.* **61** (2012) 271–275.
24. D. M. Taylor, Measuring techniques for electrostatics, *Electrostatics* **51-52** (2001) 502–508.
25. H. Martens and M. Martens, Modified jack-knife estimation of parameter uncertainty in bilinear modeling by partial least squares regression (PLSR), *Food Qual. Prefer.* **11** (2000) 5–16; DOI: [http://dx.doi.org/10.1016/S0950-3293\(99\)00039-7](http://dx.doi.org/10.1016/S0950-3293(99)00039-7).
26. A. Franc, J. Muselík, R. Máslová and J. Hadrabová, Content uniformity of warfarin-containing mixtures and tablets, *Ces. Slov. Farm.* **62** (2013) 177–181.
27. M. Perrault, F. Bertrand and J. Chaouki, An investigation of magnesium stearate mixing in a V-blender through gamma-ray detection, *Powder Technol.* **200** (2010) 234–245.
28. H. Yang, How many batches are needed for process validation under the new FDA Guidance?, *PDA J. Pharm. Sci. Technol.* **67** (2013) 53–62; DOI: 10.5731/pdajpst.2013.00902.
29. Camo software website, Classical DoE methods and PLS-ANOVA, Specific methods for analyzing designed data; <http://www.camo.com/resources/classical-doe-methods-pls-anova.html>; access date October 19, 2013.
30. Camo software. User's manual to Unscrambler X software, v.1.3.