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Original scientific paper

Optimization and statistical evaluation of discriminative dissolution method for bisoprolol immediate-release film coated tablets

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Abstract

This study presents optimization of a statistically based approach for setting up the dissolution test conditions for bisoprolol film-coated tablets using multivariate release models as predictive *in vivo* assessment tools for formulation behaviour. Additionally, the dissolution profiles of three different strengths of bisoprolol film-coated tablets were evaluated. According to the biopharmaceutics classification system, the tested medicinal product belongs to BCS Class I (high solubility, high permeability).

Three dissolution media, including the dissolution medium of choice (pH 1.2) according to the USP monograph for bisoprolol tablets and two apparatus, paddle and basket were applied. The optimal conditions for performing the dissolution test were following: 900 mL of pH 1.2 as dissolution medium, apparatus 2 (paddle) with 75 r/min stirring speed. The quantity of the released active substance was determined using HPLC method.

For a reliable statistical analysis, multivariate methods such as model-dependent approach coupled to multivariate statistics (Weibull), multivariate model-independent approach based on generalized statistical distance (Mahalanobis distance) have been applied for evaluation of dissolution profiles. All applied statistical approaches unequivocally support the underlying similarity of the pairs in different media between different strengths. Moreover, the optimized dissolution method has a discriminatory power to reflect the characteristics of the medicinal product in order to distinguish any changes related to quantitative composition of the formulation.

Keywords: bisoprolol film-coated tablet, dissolution profiles, model-independent multivariate statistical distance, model-dependent multivariate statistical distance

Introduction

Dissolution tests are used for many purposes in the pharmaceutical industry: in the development of new medicinal products, for quality control and, to predict the *in vivo* performance. The dissolution test is developed for evaluation of *in vitro* availability of solid pharmaceutical dosage forms and to provide information about the active substance release in function of time. It is required when the absorption of the active substance is necessary for therapeutic effect. Almost every monograph of solid dosage form, in official pharmacopoeias, states the conditions for performing the dissolution test. However, parameters for setting up the dissolution test should be investigated and optimized for current medicinal product

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formulation (Guidance for industry FDA, 1997; USP 38-NF 33, 2015).

Generally, in the development of dissolution test conditions for immediate release solid oral dosage form, water is used as dissolution medium. Aqueous buffer solutions or diluted hydrochloric acid can also be applied. Surfactants and electrolytes can be added to the medium with the intention of improving the solubility. Paddle is usually used as apparatus for tablets, at the stirring speed of 50 r/min or 75 r/min. Basket is usually used as apparatus for capsules or pharmaceutical forms that tend to float in the dissolution medium. In this case, the usual stirring speed is 100 r/min. The evaluation is accomplished after the end of the test, determined the percent of the active substance release in the dissolution medium (Soni et al., 2008).

The establishment of dissolution profiles is recommended as support in the development phase for determination of *in vitro/in vivo* correlation. This could be achieved if the conditions in the gastrointestinal tract were successfully reconstructed *in vitro*. Therefore, brief comments are made concerning the optimization of *in vitro* dissolution media as well as the hydrodynamics of the test. A combination of physical-chemical measurements, *in vitro* tests, *in vivo* methods, and physiology-based pharmacokinetic modelling is expected to create a unique knowledge platform, enabling the bottlenecks in drug development to be removed and the whole process of medicinal product development to become more efficient.

Regarding to the biopharmaceutics classification system (Amidon et. al., 1995), the active substance solubility profile in buffer solutions with different pH values, its pKa value and partition coefficient should be considered for setting up the dissolution test conditions (Löbenberg et al., 2000). Comparison of medicinal product dissolution profiles is recommended in three different dissolution media, in the pH range of 1-7.5 (Carvalho-Silva et al., 2004).

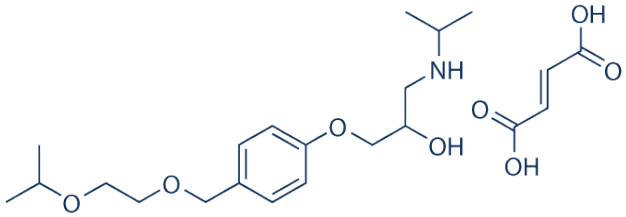


Fig. 1. Chemical structure of bisoprolol fumarate.

The evaluated medicinal product in this study, bisoprolol film-coated tablets, belongs to BCS Class I (high solubility, high permeability), thus the particle size of bisoprolol fumarate is not a critical parameter. Bisoprolol fumarate (Fig. 1) is highly beta 1-selective adrenoreceptor antagonist. The active substance has pKa values of 9.59±0.01 and molecular weight of 441.5 g/mol. The study of its solubility profile in buffer solutions of different pH values indicates that bisoprolol fumarate is a highly soluble active substance. Additionally, pharmacokinetic data regarding absorption and plasma concentration indicate that bisoprolol fumarate is a high permeability active substance (Tjandrawinata et al., 2013, https://pubchem.ncbi.nlm.nih.gov/compound/Bisoprolol-fumarate).

Several methods for evaluation and comparison of the dissolution profiles are described in the literature. Model-dependent methods consider various mathematical models to associate profiles. According to Costa and Lobo release models with major applicability and that best describe active substance release phenomena are Weibull along with Higuchi, zero-order and Korsmeyer-Peppas (Costa et al., 2001).

The aim of this study was to optimize statistically based approach for setting up the dissolution test conditions for bisoprolol film-coated tablets using multivariate release models as predictive *in vivo* assessment tools for formulation behaviour. Additionally, the dissolution profiles of three different strengths of bisoprolol film-coated tablets were evaluated.

Materials and methods

Reagents, materials and equipment

Bisoprolol film-coated tablets 2.5 mg, 5 mg and 10 mg, bisoprolol fumarate working standard (WS), potassium dihydrogen phosphate and sodium hydroxide were with analytical grade. Dissolution media: pH 1.2 (HCl, NaCl), acetate buffer pH 4.5 and phosphate buffer pH 6.8 were prepared according to directions in European Pharmacopeia monograph (Ph. Eur., 5.17., 2010).

The following instruments were used: six-station dissolution apparatus (Varian-Vankel 7025 Model: 115/230) in accordance with USP general methods, pH meter (Mettler Toledo), hotplate stirrer (IKA C-MAG HS7), analytical balance (Sartorius CPA 225D-OCE). The active substance release percent (DR %) was assayed by HPLC method at the wavelength of 227 nm, in accordance with the United States Pharmacopeia general method (USP-31- NF 26, Vol 3:3526, 2008).

Dissolution tests conditions

The dissolution tests on bisoprolol film-coated tablets were performed using Apparatus I and II at 37±0.5°, with a rotation speed of 75 r/min for paddle and 100 r/min for basket using 900 mL buffer pH 1.2 as dissolution media. After the end of test time, each sample aliquot was diluted to a suitable concentration and then analyzed by HPLC method.

Dissolution profiles conditions

Dissolution profiles for medicinal products were carried out using the most suitable dissolution conditions previously tested, and twelve (12) film-coated tablets of each dosage strength were analyzed.

Sampling aliquots of 10 mL were taken at 10, 15, 20 and 30 minutes and replaced with an equal volume of the fresh medium maintained at the same temperature. After the end of each test time, samples aliquots were filtered through 0.45 μm membrane filter (regenerated cellulose, RC) and diluted with respective dissolution medium to a suitable concentration and then analysed by HPLC method.

Dissolution profiles were obtained plotting the percent of the active substance release *vs.* time. For the statistical evaluation of the equivalence between the dissolution profiles, the dissolution efficiency was calculated and methods based on multivariate statistics were applied.

Multivariate model-independent approach based on generalized statistical distance

The model-independent MSD method was first proposed by Tsong al. (1996), and relies on the determination of the similarity limits based on inter batch differences in dissolution from tested batches or an acceptable percentage difference. Evaluation of the similarity is done after the estimation of the Mahalanobis distance between its profiles, the corresponding 90% one-sided confidence interval of true MSD. The MSD procedure (Saranadasa et al., 2005) relies on the calculation of the Mahalanobis Distance, according to Eq. (1).

$$D_{\mathrm{M}} = \sqrt{\left(\mathbf{x}_{\mathrm{T}} - \mathbf{x}_{\mathrm{R}}\right)^{\mathrm{T}} \Sigma_{\mathrm{pooled}}^{-1} \left(\mathbf{x}_{\mathrm{T}} - \mathbf{x}_{\mathrm{R}}\right)} \qquad (1)$$

Multivariate model-dependent approach

As mentioned in the introduction, this classification includes different methods of statistical comparisons (multivariate in most cases) that require fitting the dissolution curves to equations or models that represent them. In general, there is no universal model to fit all dissolution profiles, and there are no established criteria to select the proper mathematical model. To choose the best-fitting equation, average data (n=12) obtained for each strengths were fit with two statistical software packages, with Wolfram Mathematica 10 and Microsoft Excel 2007.

Nevertheless, what are the criteria to choose the ‘‘best model’’ to study active substance dissolution / release phenomena? One common method uses is the coefficient of determination, R^2, to assess the ‘‘fit’’ of a model equation. Considering to R^2 value (>0.99) the Weibull model was more useful for comparing the release profiles, according to Eq. (2).

$$F(t) = 100 \cdot \left[1 - \exp\left(-\frac{t^{\beta}}{\alpha}\right)\right] \qquad (2)$$

Subsequently, the model parameters α and β have been obtained for all available series of data by non-linear least-squares fitting procedure.

Results and discussion

Model-independent methods associate the dissolution assay results of simple ratio percent dissolved active substance (t_x %) or based on the area under the release curve (AUC) of dissolution profiles, obtained from reference and test products (Shah et al., 1998). The most adopted model-independent methods are the difference factor (f_1) and the similarity factor (f_2). Unfortunately, the obtained results have shown that *in vitro* comparative dissolution analysis using pair-wise independent-model procedures, such as difference (f_1) and similarity (f_2) factors are not suitable, because one of the requirements (not more than one mean value dissolves more than 85%, for any of the formulations) was not fulfilled (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr., 2010).

Starting from all these considerations, this work was divided in two parts. The first concerning the dissolution test set up, using buffer pH 1.2 as dissolution media, two apparatus and different rotating speed. The second part implicated in the use of the optimized dissolution test condition, obtained in the first part, to evaluate dissolution profiles of three different dosage strengths of the medicinal product.

The dissolution efficiency was calculated and all the results were statistically compared. If the comparisons of these profiles demonstrated similarity between different product dosage strengths, *in vivo* bioequivalence testing can be waived.

pKa

The active substance pKa value was determined using the potentiometric titration method. Thus, 0.01 M phosphate buffer was used to prepare the sample solution, and pH of the buffer were accurately adjusted from pH 1.9 to 11.2 with 0.2 interval using 0.5 M HCl and 0.5 M KOH titrants as appropriate.

Form the obtained data, one pKa with an average value of 9.59±0.01 was observed (Fig. 2).

Solubility

The sample intrinsic and kinetic solubility were determined using the Sirius Curve fitting solubility experiment. The sample solution was titrated from pH 4.4 to high pH with 0.5 M KOH. At a pH of 9.12, the sample precipitated from solution, as detected by a UV-turbidity

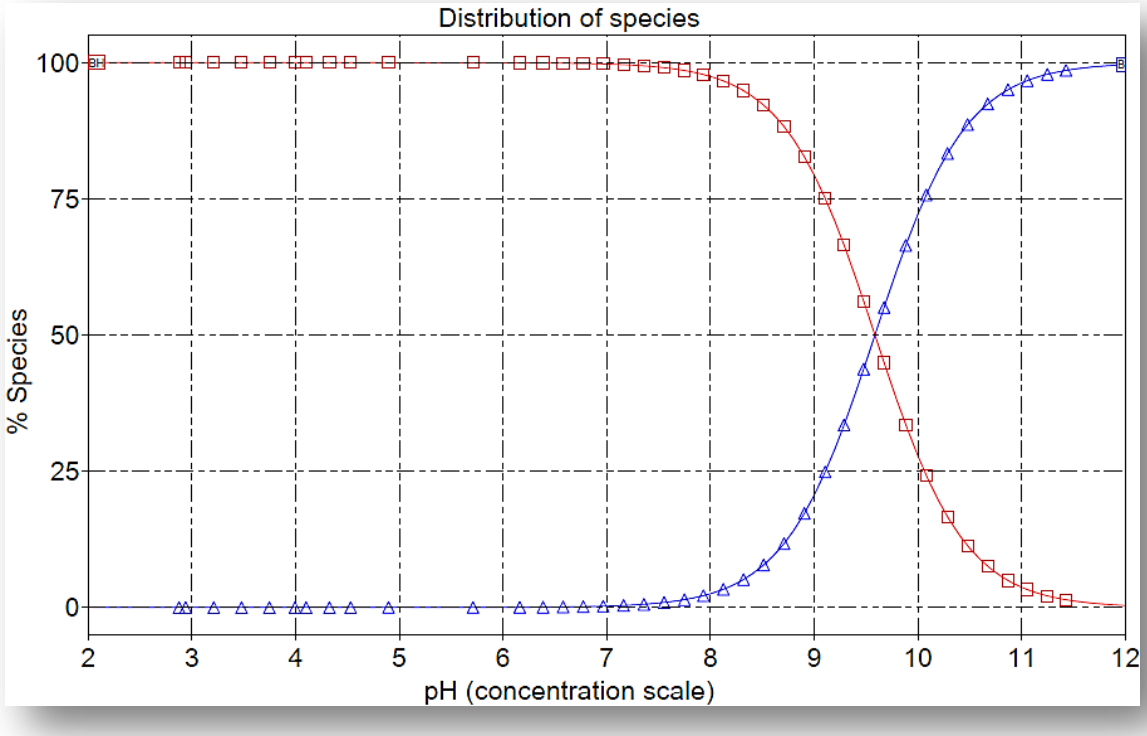


Fig. 2. Distribution of species of bisoprolol fumarate determinate with potentiometric titration method.

probe, corresponding to a kinetic solubility of 56 mM (18 mg/mL). After precipitation, the pH gradient was monitored after each addition of KOH and the pH was recorded when d(pH)/d(t) had tended to zero (i.e. where the solid and solution are at equilibrium). The precipitate Bjerrum curve thus obtained was used to calculate the sample intrinsic aqueous solubility (46.9 mM) using mass and charge balance equations. It should be noted that as the kinetic and intrinsic solubility of this compound are similar, this compound is not capable of forming significantly supersaturated solutions under the experimental conditions of this investigation (Avdeef et. al., 1982).

The sample solubility was also determined in buffer pH 1.2, using a shake-flask protocol with sample quantification by UV-spectroscopy. To enable sample quantification, the molar extinction coefficients of bisoprolol fumarate were measured at a sample concentration of 50 μM in pH 1.2. To determine the solubility, 3.0 mL of buffer pH 1.2 was added to 7.2 g of pure bisoprolol fumarate, producing a suspension that was subsequently agitated for 12 hours on an electronic shake-plate. After agitation, the sample was left for a sedimentation period of 12 hours before an aliquot of the supernatant solution was extracted by pipette, filtered under vacuum through a 0.2 μm PVDF filter plate, and its absorption spectrum recorded in pH 1.2 (diluted by a factor of 10000). The solubility was subsequently determined as 2182 mM (1674 mg/ml) from the measured absorption of the supernatant solution (over a wavelength range of 250 nm-270 nm), the experimental dilution factor, and the previously determined molar extinction coefficients (Fig. 3).

Dissolution test results

Optimization of the method included selection of suitable stirring speed in order to obtain dissolution method with required performance, providing data that are not highly variable and to avoid coning or mounding problems. On 50 r/min conning was noticed which lead to incomplete release of active substance from tablets and risk for obtaining variable results. On the other hand, when compared paddle (75 r/min) *vs.* basket (100 r/min) similar profile of active substance release was observed. Dissolution was evaluated by measuring the amount dissolved over time and carried out on twelve (12) tablets. The obtained results are presented in Table 1. The dissolution profile of bisoprolol 10 mg film-coated tablets is shown on Fig. 4.

The results from evaluation of dissolution profile using pH 1.2 as dissolution medium and paddle or basket as apparatus at the stirring speed of 75 r/min, the show no evident difference. According to the results represented in Fig. 4, it could be presumed that basket as apparatus at stirring speed of 100 r/min is equivalent to paddle as apparatus at the stirring speed of 50 r/min.

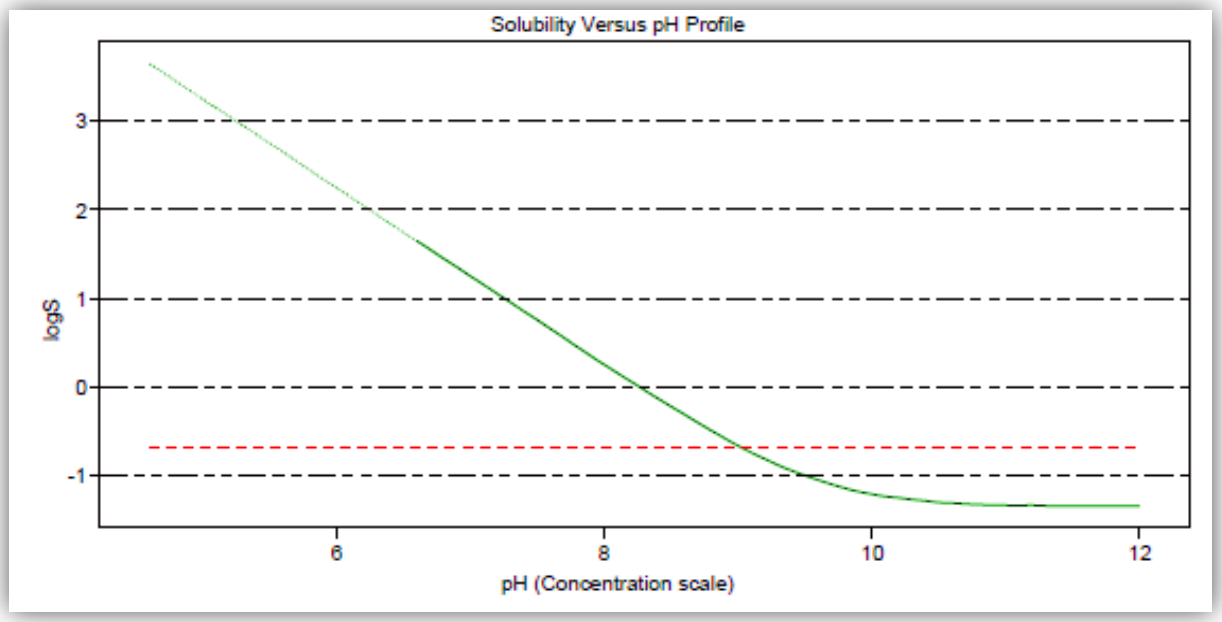


Fig. 3. Solubility *vs.* pH profile of bisoprolol fumarate.

The discriminatory power of the proposed dissolution method was confirmed by comparing the dissolution profiles for the two different formulations of Bisoprolol film-coated tablets in buffer pH 1.2 with paddle 75 r/min: original formulation and formulation with Hypromellose K (coating excipient) *vs.* proposed formulation with Hypromellose K 15 Premium K (coating excipient). Results have shown substantial differences in the dissolution profiles of the given formulations (Fig. 5).

Results reveal that inclusion of Hypromellose K 15 Premium in the formulation decreased the percent of dissolved substance from cca 100% (proposed formulation) down to cca 18% after 20 minutes. According to the above statement, the investigated dissolution conditions provide a method that is discriminating.

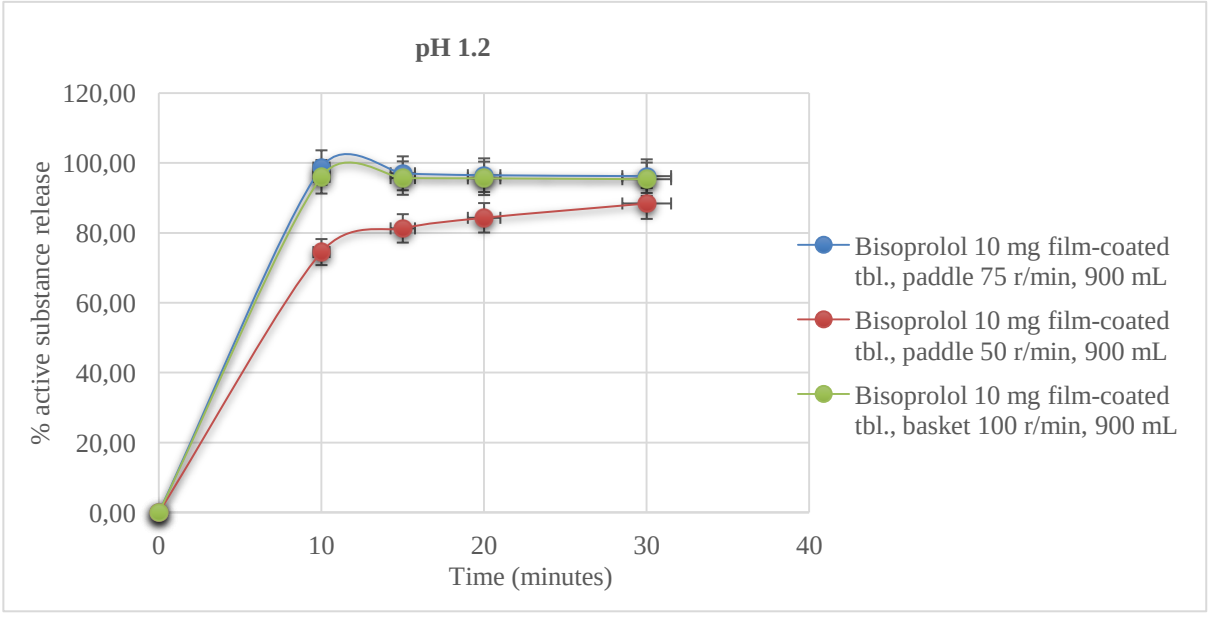


Fig. 4. Dissolution profile of bisoprolol 10 mg film-coated tablets using different apparatus and rotating speed.

Table 1. The obtained results of dissolution test of bisoprolol 10 mg film-coated tablets using different apparatus and rotating speed

	Medium pH 1.2											
	Bisoprolol 10 mg film-coated tbl. paddle, 75 r/min, 900 mL				Bisoprolol 10 mg film-coated tbl. paddle, 50 r/min, 900 mL				Bisoprolol 10 mg film-coated tbl, basket, 100 r/min, 900 mL			
Time	10’	15’	20’	30’	10’	15’	20’	30’	10’	15’	20’	30’
min.=	92.66	92.63	93.15	93.44	33.87	42.31	45.74	57.16	90.38	90.04	89.92	89.75
max.=	105.67	100.85	100.50	99.36	90.52	94.82	93.96	96.47	101.95	101.10	101.27	101.19
Average=	98.68	97.04	96.49	96.22	74.49	81.27	84.30	88.41	96.04	95.67	95.59	95.35
SD=	3.15	2.38	2.15	1.83	15.44	13.92	13.07	10.41	3.65	3.54	3.50	3.50
% RSD=	3.19	2.45	2.23	1.91	20.72	17.12	15.50	11.77	3.80	3.71	3.66	2.57

Comparative dissolution data

In order to confirm the applicability of the proposed dissolution method for all the strengths of bisoprolol film-coated tablets (2.5 mg, 5 mg and 10 mg), the dissolution profiles in 900 mL buffer pH 1.2 with paddle rotation speed of 75 r/min were compared. Results of performed comparative dissolution study are provided in Table 2 and Fig. 6. The procedures have been performed in a standard apparatus 2 with paddle at rotation speed of 75 r/min in 900 mL of above-described medium. The dissolution rate of bisoprolol fumarate has been determined using HPLC method. Similarity have been justified by dissolution profiles covering four time points obtained at specified medium buffer pH 1.2, 4.5 and 6.8. Evaluation of the dissolution profile in three different mediums was done on:

- Bisoprolol 10 mg film-coated tablet *vs.* Bisoprolol 5 mg film-coated tablet
- Bisoprolol 10 mg film-coated tablet *vs.* Bisoprolol 2.5mg film-coated tablet

The results obtained from all generated profiles, more than one mean value above 85% for any of the tested batches is observed at 15 minutes, creating a consequence that the f_2 statistics for determining profile similarity is not applicable. In accordance to the guideline (EMA, Guideline on the investigation of bioequivalence, 2010) in such cases alternative statistical methodologies can be employed for demonstrating dissolution similarity. Therefore, in order to provide a more accurate, statistically justified conclusion, analysis on the basis of model-independent method based on generalized statistical distance and model-dependent method, coupled to multivariate statistical approach were accomplished.

A multivariate confidence region procedure, based on 90% confidence intervals of the generalized statistical distance between the variables is carried out.

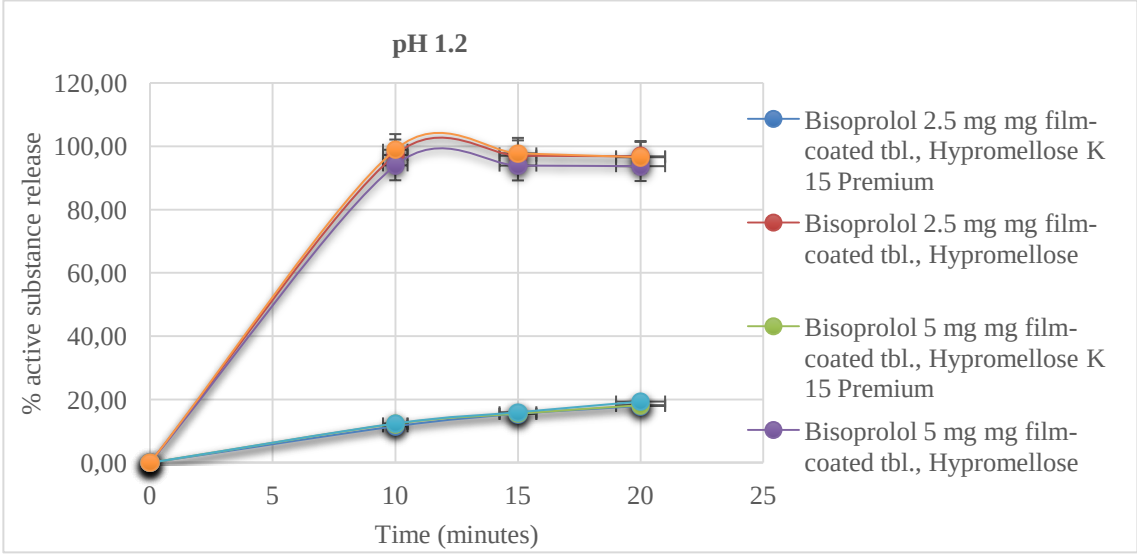
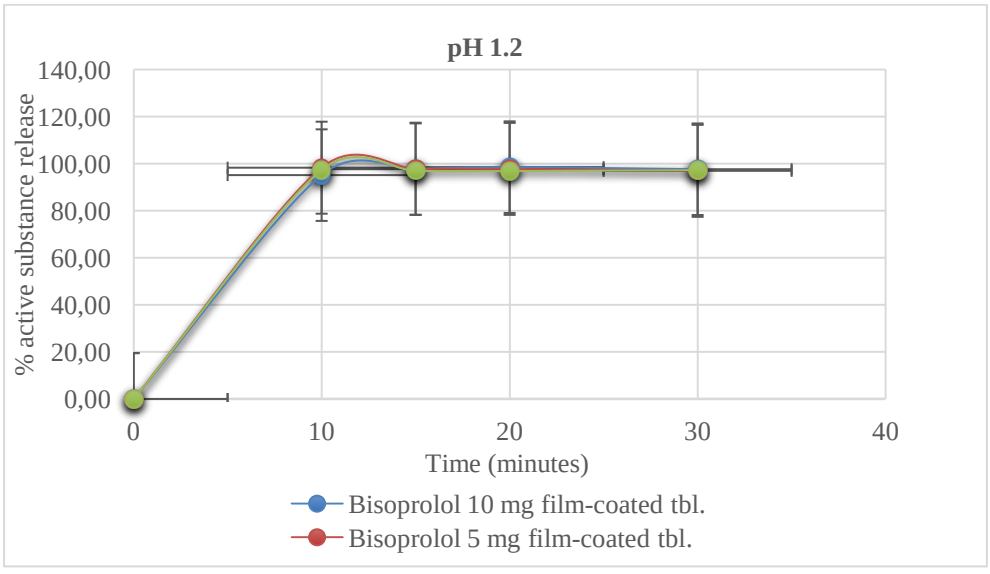
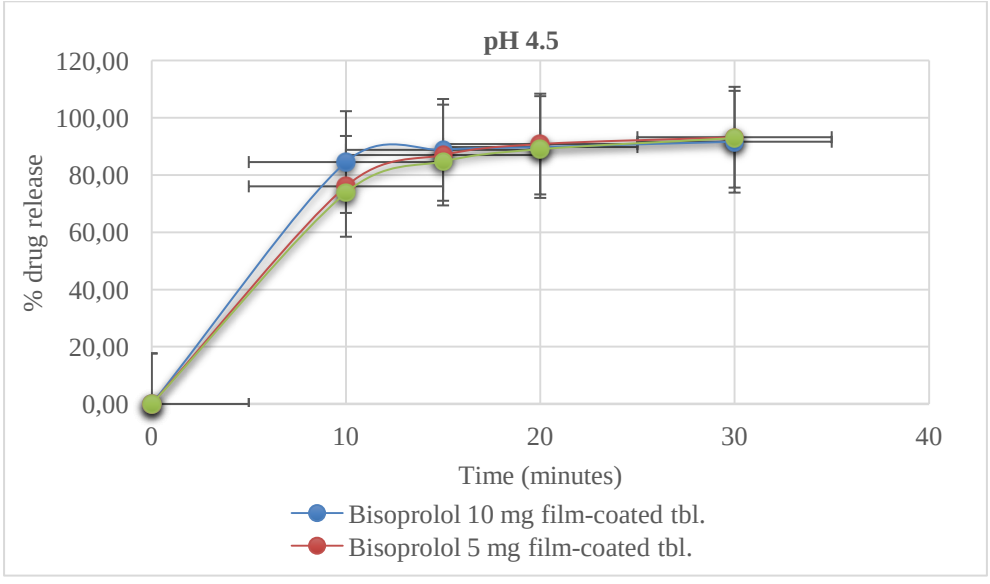


Fig. 5. Dissolution profile of bisoprolol 2.5, 5 and 10 mg film-coated tablets with a change in the type of excipients (mis-manufactured).

a)



b)



c)

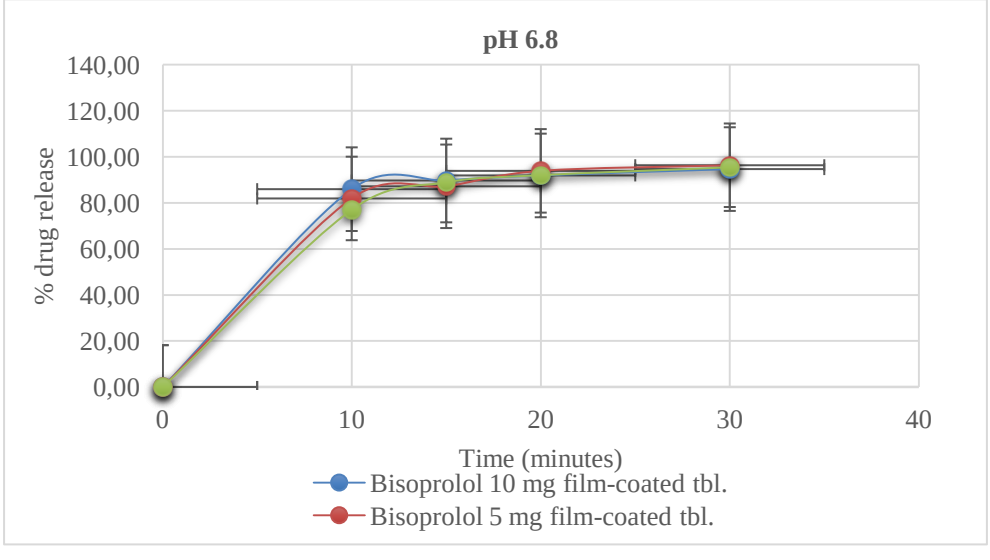


Fig. 6. *In vitro* dissolution profiles of different strengths of bisoprolol film-coated tablets: a) in medium pH 1.2, b) in medium pH 4.5, c) in medium pH 6.8.

Table 2. *In vitro* dissolution profiles of different strengths of the medicinal products

Table 2a. *In vitro* dissolution profiles of different strengths of the medicinal products in medium pH 1.2

	Bisoprolol 10 mg film-coated tbl.				Bisoprolol 5 mg film-coated tbl.				Bisoprolol 2.5 mg film-coated tbl			
Time	10’	15’	20’	30’	10’	15’	20’	30’	10’	15’	20’	30’
min.=	75.89	84.17	87.18	92.01	69.71	81.67	86.92	94.28	59.26	68.28	75.48	82.77
max.=	94.94	97.17	100.33	102.89	88.18	98.28	101.05	102.36	93.25	98.53	98.60	99.99
Average=	85.93	89.70	91.90	94.66	81.90	87.18	93.89	96.32	77.00	88.89	92.01	95.42
SD=	5.56	4.33	3.69	2.89	6.19	4.63	3.75	2.13	10.18	8.87	6.62	4.59
%RSD=	6.47	4.83	4.01	3.05	7.56	5.31	4.00	2.21	13.22	9.98	7.20	4.81

Table 2b. *In vitro* dissolution profiles of different strengths of the medicinal products in medium pH 4.5

	Bisoprolol 10 mg film-coated tbl.				Bisoprolol 5 mg film-coated tbl.				Bisoprolol 2.5 mg film-coated tbl			
Time	10	15	20	30	10	15	20	30	10	15	20	30
min.=	71.15	80.87	79.96	81.39	56.61	72.59	79.73	88.63	47.78	70.06	65.33	72.78
max.=	100.54	100.49	98.56	101.35	97.13	98.25	100.25	99.93	96.95	96.93	99.82	98.11
Average=	84.55	88.82	89.80	91.66	76.07	87.01	90.85	93.23	73.83	84.76	89.07	92.94
SD=	8.70	5.21	5.20	5.11	13.73	6.98	5.29	3.67	13.73	7.29	8.72	6.68
%RSD=	10.29	5.87	5.79	5.58	18.05	8.03	5.82	3.94	18.60	8.60	9.79	7.19

Table 2c. *In vitro* dissolution profiles of different strengths of the medicinal products in medium pH 6.8

	Bisoprolol 10 mg film-coated tbl.				Bisoprolol 5 mg film-coated tbl.				Bisoprolol 2.5 mg film-coated tbl.			
Time	10	15	20	30	10	15	20	30	10	15	20	30
min.=	75.89	84.17	87.18	92.01	69.71	81.67	86.92	94.28	59.26	68.28	75.48	82.77
max.=	94.94	97.17	100.33	102.89	88.18	98.28	101.05	102.36	93.25	98.53	98.60	99.99
Average=	85.93	89.70	91.90	94.66	81.90	87.18	93.89	96.32	77.00	88.89	92.01	95.42
SD=	5.56	4.33	3.69	2.89	6.19	4.63	3.75	2.13	10.18	8.87	6.62	4.59
%RSD=	6.47	4.83	4.01	3.05	7.56	5.31	4.00	2.21	13.22	9.98	7.20	4.81

Table 3. Mahalanobis distance between 10 mg *vs*.5mg and 2.5mg film-coated

	K	F(p,n1+ n2-p-1,0.90)	DM	90 % CI-low	90 % CI-high	DM,*max.*
			pH 1.2			
5 mg	1.2955	2.2663	1.3523	0.0296	2.6749	3.5588
2.5 mg	1.2955	2.2663	1.1128	-0.2099	2.4355	3.7819
			pH 4.5			
5 mg	1.2955	2.2663	1.2020	-0.1206	2.5247	2.4069
2.5 mg	1.2955	2.2663	1.4430	0.1203	2.7656	2.0141
			pH 6.8			
5 mg	1.2955	2.2663	3.2779	1.9552	4.6006	3.9548
2.5 mg	1.2955	2.2663	1.8335	0.5108	3.1561	2.8107

Table 4. Two-parameter Weibull model function – logarithmic (ln) transformation of data for fitting parameters between different strengths of the medicinal product

	K	F(p,n1+ n2-p-1,0.90)	DM	90 % CI-low	90 % CI-high	DM,*max.*
			pH 1.2			
5 mg	2.7329	2.5893	1.2010	0.2276	2.1744	18.4107
2.5 mg	2.7329	2.5893	0.7649	-0.2084	1.7383	18.4107
			pH 4.5			
5 mg	2.7329	2.5893	0.4218	-0.5264	1.3700	6.5615
2.5 mg	2.7329	2.5893	0.4879	-0.4603	1.4361	6.5615
			pH 6.8			
5 mg	2.7329	2.5893	2.2932	1.3199	2.6153	3.3603
2.5 mg	2.7329	2.5893	1.6420	0.6686	2.6153	3.3603

The method that is actually adopted in the present study closely follows the approach derived by Tsong et al. (1996). These authors have described a multivariate method that uses the Mahalanobis distance as a particular generalized statistical distance parameter, which takes the variability and correlation structure into account in measuring the difference between mean dissolution profiles (Table 3).

The decisions of similarity can be made with appropriate comparison of the confidence limits of the estimated metric with a prespecified similarity limit $DM,_{max}$. The 90% confidence limits of D_M can be obtained from the multivariate confidence region of the expected values of x_{test} and x_{ref} (the sample means), under multivariate normal assumption. When looking at the results from the performed multivariate method, it is obvious that these dissolution profiles can be considered as similar.

Additionally, for the purpose of the present study model-dependent approach function based on generalized statistical distance was applied. The first step of the model-dependent analysis involved selection of a suitable mathematical function to describe the dissolution data. Considering the higher determination coefficient, the preferred model that fits best to the dissolution data between strengths was the Weibull distribution model. The Weibull shape parameter, β, showed no significant variation (β>1). The results of the Weibull-multivariate statistical test are presented in Table 4.

Conclusion

The dissolution profiles of three different strengths of bisoprolol film-coated tablets were evaluated using optimized statistical approach based on multivariate release models. All applied statistical approaches unequivocally support the underlying similarity of the pairs in different media between different strengths. The preferred model that fits best to the dissolution data between strengths was the Weibull distribution model. The optimized dissolution method has a discriminatory power to reflect the characteristics of the medicinal product in order to distinguish any changes related to quantitative composition of the formulation.

The discriminating multivariate statistical approach, described in this paper, provides extra arguments when deciding if two profiles are similar, as it allows a better description of the dissolution process (i.e., rate and amount dissolved) and thus a better prediction of *in vivo* performance. According to these simulations, the established approach can be used to waive *in vivo* bioequivalence testing between different strengths form the medicinal product.

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Резиме

Оптимизација и статистичка евалуација на дискриминаторен метод на растворливост за бисопролол филм-обложени таблети со конвенционално ослободување

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Клучни зборови: бисопролол филм-обложени таблети, профили на растворливост, модел-независна мултиваријатна статистика, модел-зависна мултиваријатна статистика

Во овој труд прикажана е оптимизација на статистички базиран пристап за воспоставување на услови за определување на растворливоста на бисопролол филм-обложени таблети со примена на мултиваријатни модели за предвидување на карактеритиките на формулацијата во *in vivo* услови. Дополнително, евалуирани се и профилите на растворливост за трите различни јачини од бисопролол филм-обложените таблети. Во согласност со „Биофармацевтскиот систем за класификација'' (BCS), испитуваниот лек припаѓа на BCS класа 1 (висока растворливост, висока пермеабилност).

Испитувањето е извршено со примена на три медиуми за растворливост, вклучително и медиум со pH 1,2, што е претставен како медиум од избор во USP монографијата за бисопролол таблети и со примена на два апарати за растворливост, весло и корпа. Како оптимални услови за изведба на тестот за растворливост избрани се следните услови: 900 mL медиум за растворливост pH 1,2, апарат 2 (весло) со брзина на вртење 75 вртежи во минута. Содржината на ослободената активната супстанција е определена со примена на HPLC метод.

Евалуацијата на профилите на растворливост е извршена со примена на статистичка анализа базирана на мултиваријатни методи како што се: модел-зависниот пристап во комбинација со мултиваријантна статистика (*Weibull*) и модел-независниот метод заснован на генерализирано статистичко растојание (*Mahalanobis distance*). Сите применети статистички пристапи недвосмислено ја поддржуваат сличноста на споредените парови во испитуваните медиуми помеѓу различните јачини на лекот. Оптимизираниот метод за растворливост поседува дискриминаторна моќ да ги претстави карактеристиките на медицинскиот производ и да ги детектира евентуалните промени поврзани со квантитативниот состав во формулацијата.