ORIGINAL ARTICLE

EVALUATION OF QUALITATIVE AND QUANTITATIVE STABILITY PARAMETERS OF A NEW TABLET FORMULATION CONTAINING BISOPROLOL FUMARATE

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Abstract

Formulations that are able to modulate drug release have become an integral part of the pharmaceutical industry. The stability of the newly developed formulations depends on the physico-chemical properties of the associated substances and on various other external factors. The stability of a drug implies that its properties may change within controlled and acceptable limits. The main objective of the present study was to evaluate the qualitative and quantitative parameters of modified release tablets of bisoprolol fumarate (BF) formulated using hydroxypropyl methylcellulose and Precirol ATO5 excipients. 20 mg BF tablets were prepared through melt granulation technique using Precirol ATO5 as binder. The results of storage-stability tests showed that the obtained tablets were stable under stress condition (40 ± 2°C, RH 75 ± 5%) for at least 6 months and did not show any significant changes regarding their appearance and drug content.

Rezumat

Formulările capabile să moduleze eliberarea substanței medicamentoase au devenit o parte integrantă a industriei farmaceutice. Stabilitatea formulărilor nou dezvoltate depinde de proprietățile fizico-chimice ale substanțelor asociate, dar și de diverși factori externi. Stabilitatea unui medicament implică faptul că proprietățile sale se pot schimba în limite controlate și acceptabile. Obiectivul principal al prezentului studiului a constat în evaluarea parametrilor calitativi și cantitativi ai comprimatelor cu eliberare modificată cu 20 mg bisoprolol fumarat și excipienți precum hidroxipropil metilceluloză și Precirol ATO5. Comprimatele cu bisoprolol fumarat au fost preparate prin tehnica granulării prin topire (*melt granulation*), folosind Precirol ATO5 ca liant. Rezultatele testelor de stabilitate au demonstrat stabilitatea comprimatelor obținute în condiții de stres (40 ± 2°C, RH 75 ± 5%) cel puțin 6 luni și acestea nu au prezentat modificări semnificative privind aspectul și conținutul de substanță activă.

Keywords: bisoprolol fumarate, hydroxypropyl methylcellulose, Precirol ATO5, stability study

Introduction

Oral drug administration is the most popular drug delivery route. Innovative formulations for controlled drug release have become an essential part of oral dosage forms technology, due to their cost-effectiveness, reduced risk of systemic toxicity and of dose dumping. The stability of the newly developed formulations depends on the physico-chemical properties of the associated substances and various external factors [1]. The stability of a drug implies that its properties may change within controlled and acceptable limits. There are two ways to determine the shelf life of a drug product: experimental studies performed under normal long-term storage conditions and experimental determinations done under short-term intensive and constantly demanding conditions. One of the least complicated approaches to the manufacture of sustained release dosage forms involves embedding the drug within the retardant material, combining it with other excipients, followed by compression.

Bisoprolol fumarate (BF) is a beta blocker widely prescribed for the treatment of hypertension and angina. It is highly water soluble, with a bioavailability of 80%, due to its first pass metabolism and a clearance half-life of 3.5 h [3]. Therefore, BF requires multiple daily doses in order to maintain adequate plasma concentrations, which makes it a suitable candidate for modified drug delivery formulations [8]. Precirol® ATO5 (Prec) is a multifunctional excipient: a sustained release agent, lubricant and taste masking agent for oral dosage formulations [15]. It is suitable for melt processing techniques and is an effective problem solver in case of chemical incompatibilities. It has binding properties without affecting tablet hardness

and is not influenced by mixing/production parameters [9]. Hydroxypropyl methylcellulose (HPMC) has excellent thickening, emulsifying, film forming, protective colloid properties [10, 11]. It has been widely used in the development of new modified release formulations: mucoadhesive formulations, controlled-release pellets, microcapsules and tablets, multi-layered sustained-release tablets, coated sustained-release formulations, sustained-release ophthalmic preparations and suppositories [12]. The objective of these studies was to evaluate the qualitative and quantitative parameters of new tablets containing bisoprolol fumarate in order to acquire data on how the qualities of the drug and of the obtained dosage form undergo changes over time under the influence of various environmental factors.

Materials and Methods

Materials. Bisoprolol fumarate - 99.98% purity (BSP Unichem Laboratories LTD, India), glyceryl distearate as Precirol ATO5 (kindly offered by Gatefossé), hydroxypropyl methylcellulose (HPMC K4M, 4000 cps, Premium, Colorcon).

Methods. The preparation of 20 mg BF tablets was performed using the melt granulation technique using Precirol ATO5 as binder. [2]. The powders (bisoprolol fumarate, Precirol ATO5 and HPMC) were mixed and heated to 65°C; the obtained mass was passed through a 500 µm sieve, the granules were obtained upon cooling and further compressed on a Korsch EK0 tablet press (9 mm flat punches).

Identification and determination of the bisoprolol fumarate content

The qualitative and quantitative evaluation of the active substance in the new tablets was performed using a HPLC method with the characteristics presented in Table I.

The following solutions were prepared: the reference solution was obtained by dissolving 25 mg BF reference substance in 50 mL mobile phase; the sample solution: 20 tablets were triturated. An amount of powder corresponding to 50 mg bisoprolol was brought to 100 mL into a volumetric flask with mobile phase. After stirring for 30 minutes, 10 mL of supernatant was diluted to 100 mL with the same solvent and filtered. The bisoprolol content was calculated using the formula:

$$\mathrm{mg}\frac{\mathrm{BF}}{\mathrm{tablet}} = \frac{A_p}{A_R} * c_R * \frac{B_c}{a} * D * m * 10^{-2},$$

where: A_P & A_R = the peak areas of BF in the sample and reference solution, C_R = BF reference solution concentration (mg/mL), where B_C = content of BF reference substance (%), a = the amount of powder analysed (mg) and m = mean tablet mass (mg). Admissibility: 18.00 - 22.00 mg BF/tablet.

Table I

HPLC characteristics

Instrument	Agilent 1200 Series
Chromatographic column	Agilent Eclipse XDB-C18 150 mm × 4.6 mm, 5 µm
Column temperature	25°C
Mobile phase	pH 5.5 phosphate buffer: acetonitrile (90:10, v/v)
Flow	1 mL/min
Detection	225 nm
Automated injection volume	25 µL
Autosampler temperature	20°C

The method validation consisted in evaluating the following parameters of linearity, quantitative and detection limits, accuracy, precision and selectivity [6, 13, 14].

The analysis of related substances. The related substances were also determined using the HPLC method described above, by testing the following solutions: the sample solution was prepared as previously described. *The working solution 1*: 2.5 mL of the sample solution was diluted with mobile phase up to 25 mL, and then 1 mL was transferred into a 100 mL volumetric flask with the same solvent. *The working solution 2*: 2.5 mL of the sample solution was diluted with mobile phase up to 25 mL, and then 5 mL was transferred into a 100 mL volumetric flask with the same solvent.

Equal volumes of previously described solutions were subjected to the HPLC analysis, and the corresponding chromatograms were recorded. The elution time of the sample solution was double than the retention time of BF. The peak area of each impurity should not exceed the peak area corresponding to the working solution 1 (0.1%), and the sum of the areas of all secondary peaks should not exceed the area of the main peak obtained for the work solution 2 (0.5%) [5, 7]. The area of any secondary peak should not exceed 0.1 of the main peak area obtained for solution 2 (0.05%). The peak corresponding to fumaric acid should be disregarded.

Uniformity of dosage units. Uniformity of dosage units was evaluated according to the European Pharmacopoeia (Eur. Ph.) [4]. The drug content was determined on a representative sample by applying the procedure used for the analysis of related substances. The results were expressed as percentage of the declared content (A). The mean

tablet mass ($\overline{m}$) was determined on 10 tablets. The BF content was estimated based on the formula:

$$x_i = w_i * \frac{A}{\overline{m}}$$

The standard deviation of the contents and the acceptance value were calculated. Interpretation of the results was carried out in accordance with the Eur. Ph [4], which stipulates that the acceptability value for 10 dosage units should be less than or equal to L1 (L1 = 15). If the acceptance value is higher than L1, the determination must be repeated on 20 tablets.

Microbial contamination. Microbial contamination was evaluated according to Eur. Ph. [4] as the total number of aerobic bacteria, the total number of microorganisms, and the total number of fungi. Determination of the total number of aerobic bacteria and that of fungi was done by plate counting. Determination of specific microorganisms was performed using the selective media method. The *Escherichia coli* test was performed on 3 types of culture media according to Eur. Ph.

Evaluation of tablet stability. Long-term and short-term stability tests were performed at 25 ± 2°C, and at 40 ± 2°C and 75 ± 5% humidity, respectively, for accelerated aging, over a 3 month period, in accordance with the current legislation. For the assessment of tablets shelf-life, the long-term isothermal process was based on the drug degradation rate [7, 16]. The mathematical model of BF degradation was established based on the interpolation of experimental data.

The storage period was established through a graphical method that stated that the stability period is set by intersecting the regression equation curve with the straight line corresponding to the minimum specification provided by Eur. Ph. for drug content, or the maximum specified for the degradation products. Three series of tablets were tested.

Results and Discussion

The linearity of the HPLC method used in the study was tested over the 0.5 - 20 µg/mL range, and the calibration curve was obtained by plotting the BF concentration against the peak area. The detector response was linear with respect to BF concentration. The correlation coefficient value was 0.9983, in accordance to the acceptability criteria.

Accuracy and precision were tested by analysing six samples with theoretical concentrations of 2, 8, 15 µg/mL, with experimental results ranging from 96.75 to 103.65% for precision and 0.376 - 2.130 for accuracy, thus fulfilling the admissibility criteria [6]. Selectivity was achieved by injecting a mixture containing, besides the drug, the excipients used for compression. No significant interference was observed regarding BF.

Retention times corresponding to fumaric acid, BF, impurities A, B, C are shown in Table II. The required values for other individual impurities are max. 0.10% and the result obtained was 0.06%, within the required limits. The required total impurity value was 0.30%, which was appropriate as it was within the max. 0.50% required limits. The results obtained for the BF related impurities fell within the required limits [5, 16]. Table III presents the results obtained for the qualitative and quantitative parameters of the 20 mg BF tablets.

Table II

Retention times of BF and impurities

Component	Retention time (min)	Relative retention time (min)
Fumaric acid	1.1	0.13
Impurity A	1.5	0.17
Impurity B	2.0	0.23
Impurity C	3.4	0.38
BF	4.5	0.52

Table III

Physico-chemical and microbiological properties of the 20 mg BF tablets

Parameter	Admissibility	Results
Appearance	White, flat, uniformly shaped tablets, intact edges	Met the requirements
BF identification	The main peak on the chromatogram obtained for the sample solution must show the same retention time with the main peak in the chromatogram for the reference solution	Met the requirements
BF content (mg/tablet)	18.50 - 21.50	19.66
Related substances: - Individual, max% - Amount, max%	 0.1 0.5	 0.092 0.151
Uniformity of dosage units	According to Eur. Ph	Meet the requirements
Average weight (mg/tablet)	138.75 - 161.25	150.18

Parameter	Admissibility	Results
Microbial contamination		
- no. total aerobic bacteria/g	max. 10^3	22
- no. total aerobic fungi/g	max. 10^2	< 1
- *Escherichia coli*	absent	

The results for the long-term tests on three product series, as well as the average values of the qualitative and quantitative parameters evaluated are presented in Tables IV and V.

After12 months of storing at 25°C in PVC-aluminum packaging, the BF mg/tablet content dropped by less than 3.7% and the appearance of the tablets remained the same.

Table IV

Qualitative and quantitative evaluation results of the 20 mg BF tablets at 25°C

Parameter / Time		0 months	3 months	6 months	9 months	12 months
1st series	Description, appearance	White, round, uniformly-shaped tablets, intact edges				
	BF content (mg/tablet)	20.001	19.941	19.859	19.758	19.697
	Related substances:					
	- Individual, max%	Met the requirements	Met the requirements	Met the requirements	Met the requirements	Met the requirements
	- Amount, max%	0.148	0.198	0.231	0.273	0.320
2nd series	Description, appearance	White, round, uniformly-shaped tablets, intact edges				
	BF content (mg/tablet)	19.936	19.809	19.585	20.002	19.901
	Related substances:					
	- Individual, max%	Met the requirements	Met the requirement	Met the requirements	Met the requirements	Met the requirements
	- Amount, max%	0.149	0.201	0.235	0.264	0.321
3rd series	Description, appearance	White, round, uniformly-shaped tablets, intact edges				
	BF content (mg/tablet)	19.986	19.958	19.855	19.753	19.698
	Related substances:					
	- Individual, max%	Met the requirements	Met the requirements	Met the requirements	Met the requirements	Met the requirements
	- Amount, max%	0.147	0.202	0.233	0.284	0.325

Table V

Quantitative parameters of 20 mg BF tablets change over time

Time (months)	0	3	6	9	12
BF content (mg/tablet)	19.974	19.873	19.785	19.718	19.682
Related substances - Amount, max%	0.147	0.194	0.258	0.287	0.329
Logarithm of BF content	1.301	1.299	1.297	1.2952	1.294

By plotting the time variation against the logarithm of the concentration of bisoprolol fumarate (mg/tablet), the curve in Figure 1 shows that drug degradation is of 1st order. The delineation of the degradation was performed by experimental data interpolation, resulting in the regression curve:

$$\log c = - 0.0006 \times t + 1.3008;$$

where: c = BF content (mg/tablet) and t = time (months).

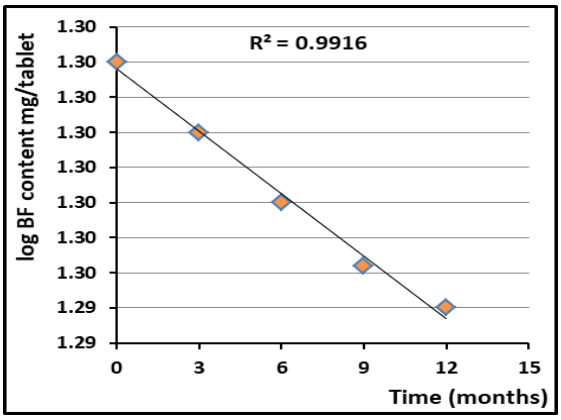

Figure 1.
Degradation curve of BF at 25°C

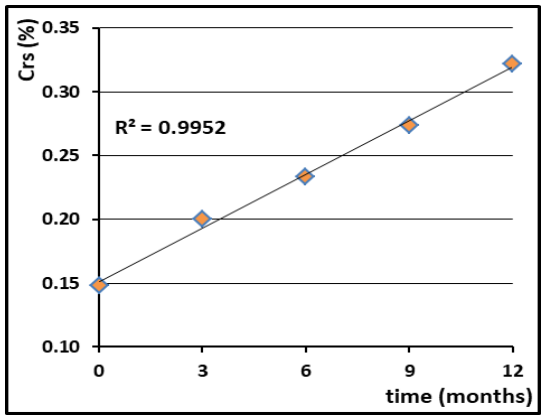

Figure 2.
Increasing content of content of related substances (C_{rs}) at 25°C

The adequacy analysis took into account 2 degrees of freedom. The experimental (y_{exp}) and calculated values (y_{calc}) for BF content based on the regression equation are shown in Table VI.

Table VI

Experimental and calculated values for BF 20 mg/tablet

X	0	3	6	9	12
y_{exp}	19.974	19.873	19.785	19.718	19.682
y_{calc}	19.965	19.845	19.789	19.709	19.678

The experimental data dispersion due to the error of experience (s_1^2) and the values calculated based on the mathematical model (s_2^2) were: s_1^2 = 0.002612 and s_2^2 = 0.000523. The Fischer test value was:

$$F_{calc} = \frac{s_2^2}{s_1^2} = 0.20.$$

The critical value for the Fischer distribution for a 95% accuracy was F (95%, 2.2) = 19. Because F_{calc} < F (95%, 2.2) it was proved that the chosen model was suitable for a 95% confidence interval [9, 11]. The content of related substance (C_{rs}) was expressed as the sum of all peak areas for related substances, max. % may be assimilated after a zero order kinetics described by the equation obtained on the curve of Figure 2.

$$C_{rs} = 0.014 \times t + 0.1511;$$

where: C_{rs} = related substances content and t = time (months).

Analysing the graphs shown in Figure 3, the stability period of the 20 mg BF tablets stored in PVC-aluminium packaging at 25°C, could be assessed at 24.1 months for a 95% probability and for an 18.5 mg/tablet BF minimum content.

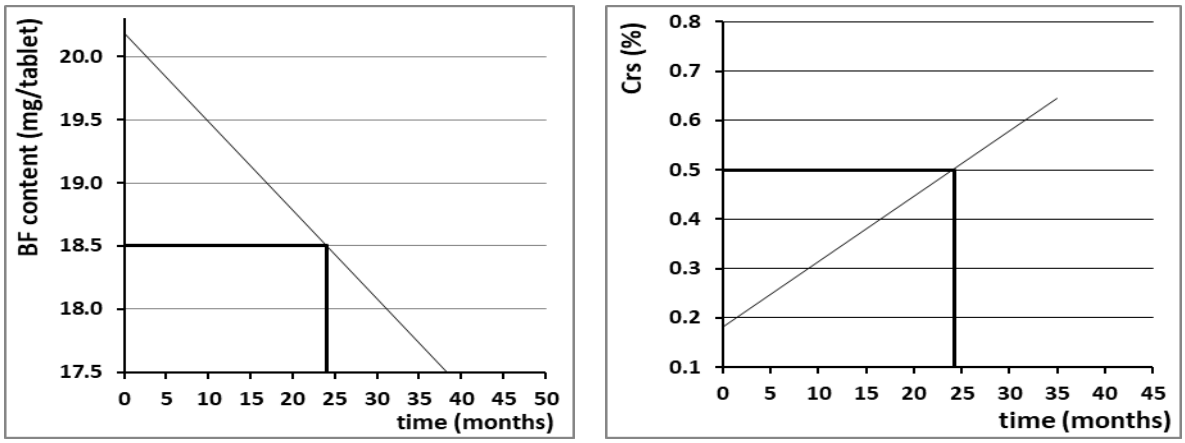

Figure 3.

Graphical evaluation of the shelf life of BF tablets at 25°C

The results of the isothermal test at 40 ± 2°C and 75 ± 5% relative humidity for 6 months on three product series are shown in Tables VII and VIII. Under these conditions, the BF content drops by maximum 6.45%. The appearance of the tablets did not change during the preservation period.

Table VII

Qualitative and quantitative evaluation of 20 mg BF tablets at 40°C

Parameter / Time		0 months	1 month	3 months	6 months
1st series	Description, appearance	White, round, uniformly-shaped tablets, intact edges			
	BF content mg/tablet	20.020	19.831	19.639	19.358
	Related substances:	Met the requirements	Met the requirements	Met the requirements	Met the requirements
	- Individual, max%	0.285	0.303	0.333	0.404
	- Amount, max%				
2nd series	Description, appearance	White, round, uniformly-shaped tablets, intact edges			
	BF content mg/tablet	19.989	19.842	19.515	19.320
	Related substances:	Met the requirements	Met the requirements	Met the requirements	Met the requirements
	- Individual, max%	0.279	0.305	0.375	0.424
	- Amount, max%				
3rd series	Description, appearance	White, round, uniformly-shaped tablets, intact edges			
	BF content mg/tablet	20.006	19.918	19.615	19.223
	Related substances:	Met the requirements	Met the requirements	Met the requirements	Met the requirements
	- Individual, max%	0.283	0.299	0.331	0.383
	- Amount, max%				

Table VIII

Changes of quantitative parameters of the 20 mg BF tablets as average values over time

Time (months)	0	1	3	6
BF content (mg/tablet)	20.002	19.796	19.579	19.251
Related substances amount (max%)	0.282	0.302	0.345	0.402

By interpolation of experimental data the following regression equations were obtained (Figure 4 and 5) for the decrease of BF content:

$$C = 0.0083 \times t^2 - 0.1209 \times t + 9.9985;$$

where: C = BF content (mg/tablet), and t = time (months) and for C_{rs}:

$$C_{rs} = 0.02535 \times t + 0.277;$$

where: C_{rs} = content of related substances, (max%), and t = time (months).

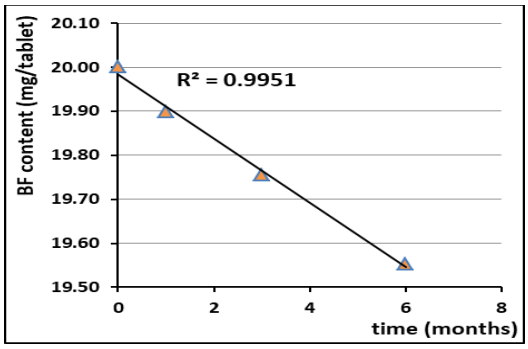

Figure 4.
BF Degradation curve at 40°C

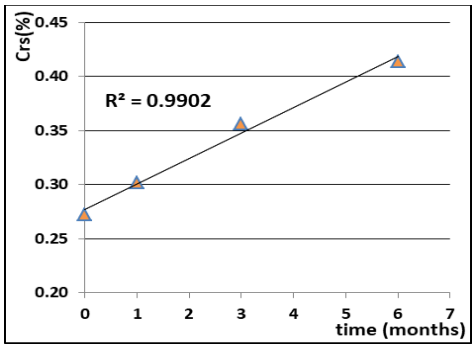

Figure 5.
Related substances content increase at 40°C

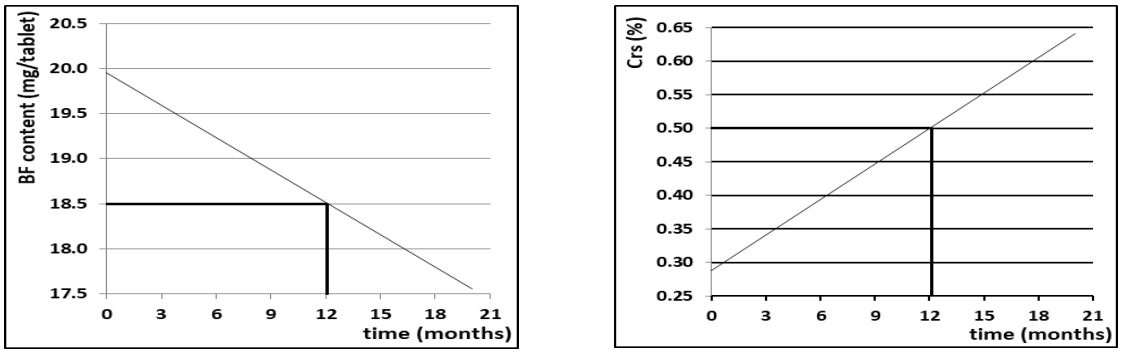

Figure 6.
Graphical evaluation of the stability period of BF tablets at 40°C

The results of the graphical stability evaluation of 20 mg BF tablet over a 6-month period are shown in Figure 6 for 18.5 mg BF/tablet minimum content and maximum content of related substances 0.05%. By analysing the graphs it was established that the stability period of the new BF tablets, stored in PVC-aluminium packaging at 40°C and 75 ± 5% relative humidity, was about 24 months. The results obtained in accelerated test conditions confirmed the data set in real time.

Conclusions

In the present study, the qualitative and quantitative parameters of BF sustained-release tables were evaluated using a HPLC method. The results obtained for the BF related impurities fell within the required limits and were appropriate. The stored tablets did not show any significant change in appearance and drug content throughout the tested stability period. Furthermore, the newly developed tablets were stable for at least 6 months under stress conditions. Stability tests were carried to ensure that the tablets were stable throughout their shelf life in accordance with ICH requirements.

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