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SUITABILITY OF AN ANALYTICAL METHOD FOR METOPROLOL TARTRATE COATED TABLET

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ABSTRACT

The suitability of an analytical method determines the characteristic performance of a method when it evaluates parameters such as selectivity, accuracy, precision and robustness. Thus, this research aims at describing the suitability of an analytical chromatographic method to determine metoprolol tartrate content in a coated tablet. This method has been considered accurate and robust since its results were recorded according to the recommended acceptance criteria (98-102%). This method was also considered precise as the results were close to two precisions to metoprolol tartrate (on 1st and 2nd days) and achieved the acceptance criteria established for such parameter. Besides, no peak degradation product coeluted with metoprolol peak, and all samples submitted to stress showed spectral purity regarding this peak. Finally, the analytical method has been considered selective, accurate, precision and robust to analyze metoprolol tartrate content.

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INTRODUCTION

Metoprolol [1-isopropylamino-3-4-(2-methoxyethyl) phenoxy] propane-2-ol] is a selective β1 adrenergic receptor antagonist, with an asymmetric center in its chain (Figure 1). Its intake occurs by a racemic mixture form, and it is used to treat systemic arterial hypertension, myocardial infarction and heart failure (Emam *et al*., 2020; Mostafavi and Foster, 2000). Initially, this treatment results in an increase in peripheral vascular resistance that, during its long-term administration, is normalized or, in some cases, reduced. It can be observed some decrease on blood pressure with metoprolol, which seems to be associated to the gradual decrease in total peripheral resistance. Metoprolol also provides some decrease on frequency and severity of ischemic events in patients with angina diagnosis, and it increases physical work capacity (Sweetman *et al*., 2007). Also, it can be supposed that the decreased demand for myocardial oxygen, which occurs in response to reduced heart rate and myocardial contractility, and may lead to this beneficial effect. In patients with supraventricular tachycardia, atrial fibrillation, ventricular extra-systoles or other ventricular arrhythmia, metoprolol has a regulating effect on heart rate. Its antiarrhythmic activity is mainly due to the inhibition of pacemaker cells automaticity and the extension of atrioventricular condition (Regardh and Johnson, 1980). Regarding its pharmacokinetics, metoprolol absorption is almost concluded after oral administration, although its bioavailability is low, about 40%, due to its significant pre-systemic elimination (Goodman, 2006). Metoprolol is mainly eliminated by hepatic metabolism due to some reactions of hydroxylation (α-hydroxymetoprolol) and demethylation (o-dimethylmetoprolol) and only 10 ± 3% of the administered drug is unchanged in urine (Cerqueira *et al*., 2003). Validation is, naturally, a basic requirement to ensure quality and reliability of the results for all analytical applications. Every laboratory involved on a qualified system needs to ensure that its analyses are suitable, accurate and precise (Fraga *et al*., 2012). The relevance to demonstrate the quality of chemical measurements has been increasingly recognized and demanded. While unreliable analytical data can lead to disastrous decisions and irreparable financial losses (Ribani *et al*., 2004). Thus, a method must undergo validation during daily operations of a laboratory to ensure its applicability and scope (Ribeiro *et al*., 2008). Brazilian Health Authority (ANVISA) requires analytical methodology validation, since it has established official documents that are guidelines to be adopted during validation process, such as RDC No. 166, July 24th, 2017, which provides for the validation of analytical methods. The International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical Products for Human Use (ICH) was launched in 1990 and emerged as a forum for a constructive dialogue between regulatory and industry authorities in order to harmonize the new drug presentation requirements among Europe, the United States of America and Japan. One of the first issues within the "Quality" section was analytical validation. Consequently, ICH was very helpful in harmonizing terms

and definitions as well as determining basic requirements (ICH, 1996). In Brazil, the pharmaceutical industry must be ruled by the Resolution of the Collegiate Board of Directors (RDC) No. 166, on July 24th, 2017, which requires that, depending on the category of the test, it has some minimum evaluation requirements during the method validation. Therefore, this paper aims at carrying out the analytical method suitability to determine metoprolol in 100-mg tartrate coated tablet using high performance liquid chromatography (HPLC) to verify the analytical method applicability described in the monograph of the 5th edition of the Brazilian Pharmacopoeia.

MATERIAL AND METHODS

Reagents and Equipment: Metoprolol tartrate standard (USP); Impurity C standard (4-[2-Hydroxy-3-[(1-methylethyl) amino] propoxy] benzaldehyde) (LGC); metoprolol tartrate raw material (IPCA); metoprolol tartrate 100 mg final product (Prati Donaduzzi); placebo (Prati-Donaduzzi). Methanol and acetonitrile (Biograde), hydrochloric acid and hydrogen peroxide (Neon), sodium 1-pentanesulfate monohydrate, sodium acetate anhydrous and iron III chloride hexahydrate (Synth), acetic acid and sodium hydroxide (Scharlau). 0.22-µm PVDF and PET membrane filters (Chromafil®). Mettler Toledo XP26 analytical balance; Elmasonic S450/(H) ultrasonic bath; Quimis Q317M drying oven; Ethik 424-CF photostability chamber; Liquid Chromatograph equipped with SPD-M20A diode array detector, LC-20AT pump (Shimadzu) and Empower software, Version 3, Feature Release 2 for integration and processing.

Analytical Methodology

Chromatographic System: The chromatographic system was based on the use of a mobile phase that consists of a mixture of 961-mg sodium 1-pentanesulfate monohydrate, 82-mg anhydrous sodium acetate, 550-mL methanol, 470-mL ultra-pure water and 0.57-mL acetic acid; isocratic mode; 1.00 mL/min flow rate; 20-µL injection volume; room temperature and 254-nm wavelength. Separation was performed on Akzo Nobel Kromasil C18 chromatographic column 250 mm x 4.6 mm x 5 µm.

Preparation of Samples

Standard Solution: 10 mg of metoprolol tartrate standard was transferred to a 10-mL volumetric flask, added to about 5-mL of methanol diluent and 0.1-M hydrochloric acid (50:50) and taken to ultrasound equipment until complete standard solubilization. From this solution, a 5-mL sample was transferred to a 10-mL volumetric flask and the volume was filled up with a mobile phase.

Trial Solution: Twenty tablets were triturated, and the amount of powder was equivalent to 50-mg of metoprolol tartrate and transferred to 50-mL volumetric flask, added to 30-mL of methanol diluent and 0.1-M hydrochloric acid (50:50) and taken to an ultrasound equipment for 30 minutes. From this solution, 5 mL were transferred to a 10-mL volumetric flask and the volume was filled up with a mobile phase.

Parameters to be assessed: The compendial method has been validated in accordance with the parameters required by RDC legislation No. 166, on July, 24th, 2017. According to Article 7, compendial analytical methods must have their suitability demonstrated for the intended use, under the laboratory operational conditions, by a validation study presentation in order to evaluate at least precision, accuracy and selectivity parameters, except for cases regarding the quantification of impurities, which must include the limit of quantification.

Selectivity: Analytical method selectivity, according to RDC No. 166 (2017), shall be shown by means of its ability of identifying or quantifying the analyte of interest undoubtedly in the presence of components that may be found out in the sample, such as impurities, diluents and matrix components.

The method selectivity was evaluated by means of:

a) evidence that the peaks quantified in the trial solution chromatogram in fact refer to the analytes of interest (metoprolol tartrate) by comparing the retention times of these peaks with their respective peaks obtained in standard solutions prepared with chemical reference substances (CRS);
b) evidence that no peak of degradation product and/or excipient coeluated with metoprolol tartrate peak in the trial solution by the analysis of the finished product, raw material and placebo samples submitted to acid, base, oxidation, metal ions, heat, light and moisture stress conditions, subsequently to verify spectral purity of metoprolol tartrate peak in these solutions. Thus, to ensure the selectivity of the method, the samples of raw material, finished product and placebo were submitted to forced degradation under the conditions mentioned in Table 1.

Precision: According to RDC No. 166 (2017), precision must evaluate how close the results obtained by assays are from one another, and the samples prepared as the description of the analytical method to be validated. Precision must be expressed by intermediate precision or reproducibility. Repeatability method must be carried out with samples under the same operational conditions, the same analyst and the same instruments, in a single analytical run. It must use at least nine (9) determinations covering linear interval of analytical method, that is, three (3) concentrations: low, medium and high, with three (3) replications in each level or six (6) replications at one hundred per cent (100%) of the test concentration prepared individually. Intermediate precision must express how close the results obtained from the analysis of the same sample, in the same laboratory, in at least two different days, carried out by different operators; and the same concentrations and the same number of determinations described in the repeatability evaluation are included.
The precision (repeatability) of the methodology was evaluated using six replications at 100% of the test concentration (500 µg/mL). The tests were validated based on relative standard deviation (RSD) analysis among the samples. The defined specification for RSD of repeatability is at most 1.9%. Intermediate precision was performed by carrying out the same analysis the next day by another analyst and comparing the results of both days. The defined specification for relative standard deviation of intermediate precision is 3.0% maximum.

Accuracy: Accuracy of an analytical method must be reached by the level of conformity among individual results of the method under test in relation to a value accepted as true. Accuracy shall be checked based on at least nine (9) determinations, including the linear interval of analytical method, that is, three (3) concentrations: low, medium and high, with three (3) replications in each level. For the final product, it is required to add a known amount of chemical reference substances (CRS) to the matrix. The relationship between the experimentally determined mean concentration and the corresponding theoretical concentration must express accuracy. The specifications defined for accuracy recovery and relative standard deviation are 98-102% and 1.9%, respectively.

Robustness: The robustness of an analytical method is its capacity to resist small and deliberate variations of the analytical parameters. It indicates its reliability during normal use. The robustness assessment must be considered during the suitability of this methodology. When susceptibility to analytical variations is observed under the tested conditions, they must be controlled and precautions must be included in the analytical methodology to guarantee their control during its application along the daily activities at the laboratory. The robustness of the studied method was evaluated by varying the main analytical conditions. Regarding the column robustness parameter, and a second column was tested from a different manufacturer (Phenomenex Luna); while for the filter, PET and PVDF 0.22-µm filters were tested; the times of 10 and 35 minutes were tested for extraction time,

and for stability of analytical solutions, it was decided to carry out the robustness within 72 hours. Finally, specifications for robustness were the same as accepted for accuracy.

RESULTS AND DISCUSSIONS

Previously, to each analysis, the system suitability was verified in accordance with the analytical methodology to prove that all chromatographic parameters were satisfactory, once they filled up resolution, tailing factor, number of theoretical plates and specified relative standard deviations in the analytical method. Such assessment was also carried out to control injections to ensure that the system remained constant during and until the end of the analysis.

Table 1. Stress conditions and time of exposure of metoprolol tartrate

Sample	Stress conditions	Time of exposure
Control	-	-
Acid stress	HCl 2.0 mol/L	10 days
Alkaline stress	NaOH 2.0 mol/L	10 days
Oxidative stress	H_2O_2 3 %	13 days
Metal ions stress	$FeCl_3$ 10 mMol/L	24 hours
Photolytic stress	2.4 million lux/h	Enough time for 2 cycles
Moisture stress	75% U.R./40ºC	10 days
Thermal Stress	60ºC	10 days

Table 2. Results of API and final product solutions in acid, basic, oxidative and metallic ion stress

Sample	Stress Condition	Content	Recovery
API	Control	99.22	-
	HCl 2.0 M	98.58	99.35
	NaOH 2.0 M	87.80	88.49
	H_2O_2 3 % (m/v)	98.21	98.98
	$FeCl_3$ 10 mM	101.21	102.01
Final Product	Control	98.10	-
	HCl 2,0 M	98.62	100.53
	NaOH 2,0 M	90.23	91.98
	H_2O_2 3 % (m/v)	98.27	100.17
	$FeCl_3$ 10 mM	99.32	101.24

Table 3. Results of API and final product solutions in conditions of physical stress

Sample	Stress Condition	Content (%)	Recovery (%)
API	Control	101.45	-
	Photolytic	100.78	99.34
	Temperature	99.71	98.28
	Moisture	99.81	98.38
Final Product	Control	99.89	-
	Photolytic	100.54	100.65
	Temperature	96.26	96.37
	Moisture	95.98	96.09

Selectivity: An impurity C standard solution and metoprolol tartrate standard solution were also prepared to prove the specificity of the proposed method regarding its specific impurity. So, the retention time (3.7 min) of impurity C was different from the retention time of metoprolol tartrate API (5.9 min), evidencing no risk of co-elution of impurity with API (Figure 1). Furthermore, this method showed selectivity for diluent and formulation components as there was no peak in the time of retention of the studied compound when placebo solution and diluent solution were injected. The stability of metoprolol succinate, under acidic and oxidative conditions, was described by Shaik and Patil (2014), Madhukar and Kannappan (2015) and Thakker *et al*. (2012). Table 2 shows the obtained quantitative data from the selectivity assay. There was no formation of degradation product in 24 hours for metal ion stress, as well as no reduction was higher than 10% of the active substance content. In literature, no stress studies were carried out on metal ions, however, since peak purity was achieved, it is understood that the absence of specialized literature does not cause any problem to the performed evaluation. Among the chemical stress essays, the basic stress essay was the one that presented the greatest reduction of API content, both in raw material and final product. Nevertheless, metoprolol peak presented spectral homogeneity in both samples. According to data on Table 2, it was observed a decrease in API content of nearly 11% for raw material and 8% for final product. The data in literature corroborate with data obtained in this study. Shaik and Patil (2014) obtained a decrease in 8.2% API content after one hour of essay (60°C) by using 2.0 M NaOH. According to Borkar *et al*. (2012), the main formed and identified degradation products in basic medium were obtained by C-O bond cleavage. Furthermore, it is worthwhile to mention that the studies about degradation, described in scientific literature (Shaikh and Patil, 2014; Yunoos *et al*., 2015), did not find out and/or obtain insignificant rates of forced degradation products in alkaline medium. These data are consistent with those ones obtained experimentally in degradation analysis in a basic medium. As it is shown on Table 2, the stability of metoprolol tartrate molecule was observed when compared to acid hydrolysis, in which the decrease in the active ingredient content was less than 1% for both raw material and final product. Shaikh and Patil (2014) also reported the same behavior in their research, since they have not seen any significant decrease (0.8%) in metoprolol succinate content. Another result was observed regarding oxidative stress, because the degradation of the active ingredient content was nearly 1%. Shaikh and Patil (2014) did not observe a significant decrease in metoprolol content (2.1%) either. Madhukar and Kannappan (2015) obtained a 2.16%-degradation of the active content, even though they have carried out the trial with the highest concentration (30% m/v) of the H2O2 stressing agent during 10 hours. Regarding physical stresses, both final product and raw material seemed to be stable, as it can be seen in Table 3. For photolytic stress, there was no formation of degradation products in both raw material and final product. Shaikh and Patil (2014) obtained an API content of 99.0% after 1.2 million lux/hour (corresponding to 1 cycle of photostability). And, in relation to the thermal stress, it was observed a 1.74% decrease of the active ingredient content for the raw material and 3.63% for the final product. Thakker *et al*. (2012) obtained a decrease of 0.58% of the active ingredient content after 24 hours of essay at 105ºC, as well as Shaikh and Patil (2014), who did not report significant degradations (0.3%) either, even after a stress of 24 hours at 60°C. Finally, the final product showed 3.9% of content decrease when submitted to moisture stress, but no increase in the sum of unknown impurities was observed. Thakker (2012) reported a 0.43% degradation of metoprolol content, considering the assay with the same relative humidity (75%), but with only 24-hour exposure. All obtained samples, submitted to physical stresses, showed spectral purity for metoprolol.

Precision: Regarding sample preparation, recovery and DPR results are shown in Table 4. According to the obtained results, individual values, recovery means and the relative standard deviation (RSD%) among metoprolol tartrate samples, the evaluated concentrations are in accordance with the established acceptance criteria for intermediate precision and repeatability. Thus, it is concluded that the method is precise since the results are near one another of both precisions for metoprolol tartrate (1st and 2nd day), so, they fulfilled the acceptance criteria established for the studied parameter.

Accuracy: The accuracy allows determining proximity between the experimental results and the actual values of the analyte present in the sample, evaluated by recovery. Samples were evaluated at three levels to determine the accuracy of the method: low (400 µg/mL - 80%), medium (500 µg/mL - 100%) and high (600 µg/mL - 120%). The recovery results of the samples are shown in Table 5. According to the above results, it is evident that both individual values and recovery means for metoprolol tartrate in the evaluated concentrations are in accordance with the acceptance criteria recommended in the accuracy parameter (98-102%). Thus, the analytical method is characterized as accurate at the proposed concentration levels for metoprolol tartrate of 100-mg coated tablet.

Table 4. Results of precision essays

Level (%)	Content 1st day (%)	Content 2nd day (%)	Means (%)	RSD (%)	Specification (%)
100	97.55	99.69	99.12	0.86	≤ 3.0
	100.35	99.74			
	99.44	99.42			
	98.06	99.15			
	97.83	99.42			
	99.23	99.66			

Table 5. Results of accuracy essays

Level	Recovery (%)	Means (%)	Specification (%)	DPR (%)	Specification (%)
80 %	101.89	101.90	98 - 102	0.05	≤ 1.9
	101.85				
	101.96				
100%	99.21	98.44		0.68	
	98.12				
	98.00				
120%	100.87	100.64		0.58	
	99.98				
	101.06				

Table 6. Quantitative results for column robustness

Conditions	Samples	Contents (%)	Means (%)	Recovery from the original condition (%)	Specification (%)
Original Column	Test Solution 01	99.86	99.69	99.97	98 - 102
	Test Solution 02	99.52			
Column Phenomenex Luna	Test Solution 01	99.95	99.66		
	Test Solution 02	99.36			

Table 7. Filter robustness for standard and sample solutions of metoprolol tartrate

Solution	Procedure	Area (µV.sec)	Recovery (%)	Specification (%)
Standard-Aliquot 01	PVDF – 0 mL of discard	581,277	99.88	98-102
Standard-Aliquot 02	PVDF - 1 mL of discard	581,561	99.93	
Standard-Aliquot 03	PET – 0 mL of discard	581,755	99.96	
Standard-Aliquot 04	PET - 1 mL of discard	582,067	100.01	
Sample-Aliquot 01	PVDF – 0 mL of discard	600,798	100.10	98-102
Sample-Aliquot 02	PVDF - 1 mL of discard	605,075	100.81	
Sample-Aliquot 03	PET – 0 mL of discard	600,483	100.04	
Sample-Aliquot 04	PET - 1 mL of discard	600,848	100.11	

Table 8. Quantitative evaluation of robustness of extraction time

Conditions	Content (%)	Mean Content (%)	Recovery (%)	Specification (%)
No change	99.69	99.57	-	98-102
	99.45			
Extraction time 10 min	99.17	98.85	99.28	
	98.53			
Extraction time 35 min	99.56	99.78	100.21	
	100.00			

Table 9. Evaluation of solution stability

Phase	Standard solutions	Sample solutions	Mean Content (%)	Recovery (%)	Specification (%)
1st	Standard 1	Test solution 1	99.53	-	98 - 102
	Standard 2	Test solution 2			
2nd	Standard 3	Test solution 3	99.93	100.40	
	Standard 4	Test solution 4			
	Standard 1	Test solution 3	100.47	100.94	
	Standard 2	Test solution 4			
	Standard 3	Test solution 1	100.43	100.90	
	Standard 4	Test solution 2			

Figure 1. Chromatogram of standard metoprolol tartrate solution and standard solution impurity C

Robustness: Analytical changes were quantitatively compared to the unchanged method by calculating metoprolol tartrate recovery in the test solution. Such evaluation refers to robustness proof in accordance with the accuracy criteria, showing that the proposed change does not result in a difference to quantify analytes.

Column Robustness: It can be observed on Table 6 the results obtained for the test solutions in both evaluated columns: the original method column (Akzo Nobel Kromasil) and the changed condition column (Phenomenex Luna). The recovery is included within the proposed acceptance criterion (98–102); therefore, columns seemed to be equivalent. The qualitative impacts of the changes were evaluated in the Test Solution - Basic Degradation. The times of retention, tail factor and number of theoretical plates of metoprolol tartrate for the method with original column and column Phenomenex Luna were 5.713 and 6.771 min, 1.78 and 1.59, and 4,848 and 4,470, respectively. The analyte peak of interest was shown spectrally pure for raw material and final product in both applied columns. It was evidenced that the change in the column model did not impair the ability of the method to quantitatively and qualitatively determine API. Thus, we can state that the method is robust for Phenomenex Luna column application.

Filter and Extraction Robustness: Table 7 shows data related to the analysis under filter robustness for standard and test solutions, where the described aliquots referred to the volume of discard and used filter. It can be observed that the analyte recovery met the specification, ensuring that this method is robust to the different evaluated filtration procedures. Thus, all the evaluated filters and discards can be applied during the analytical routine. Table 8 presents the data related to the analysis in the condition of extraction robustness. The impact on reduction (10 minutes) and increase (35 minutes) of ultrasound time on sample preparation were evaluated by comparing to the conditions described in the method (30 minutes). It can be observed that API recovery of the active under test-solutions on changed conditions has reached the specified range when compared to the condition proposed in the analytical method. This pointed out, therefore, that the proposed method is robust according to the conditions of tested extraction.

Stability of Solution: The stability of the analytical solutions was evaluated by analyzing the samples content after 72 hours under normal storage conditions (room temperature). Table 9 reports the results obtained in the first and second stages of inspection. It can be observed that the dosage obtained for the test solution that was analyzed after 72 hours from its preparation (using new standard solutions for quantification) is according to the acceptance criteria. Thus, it is indicated the stability of the test solution.

CONCLUSIONS

According to the experimental results and evaluation of scientific literature, it is possible to ensure that metoprolol tartrate molecule has great stability. No peak of degradation product co-eluted with metoprolol peak, and all samples submitted to the stresses have shown spectral purity for the peak. Besides, based on chromatograms evaluation for the applied diluents, no peaks affected the selectivity of the studied method. Thus, the studied analytical method is considered selective and an indicative of stability to analyze metoprolol tartrate content. Finally, it can be concluded that the proposed method is suitable for dosage purposes in 100-mg metoprolol tartrate of a coated tablet.

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