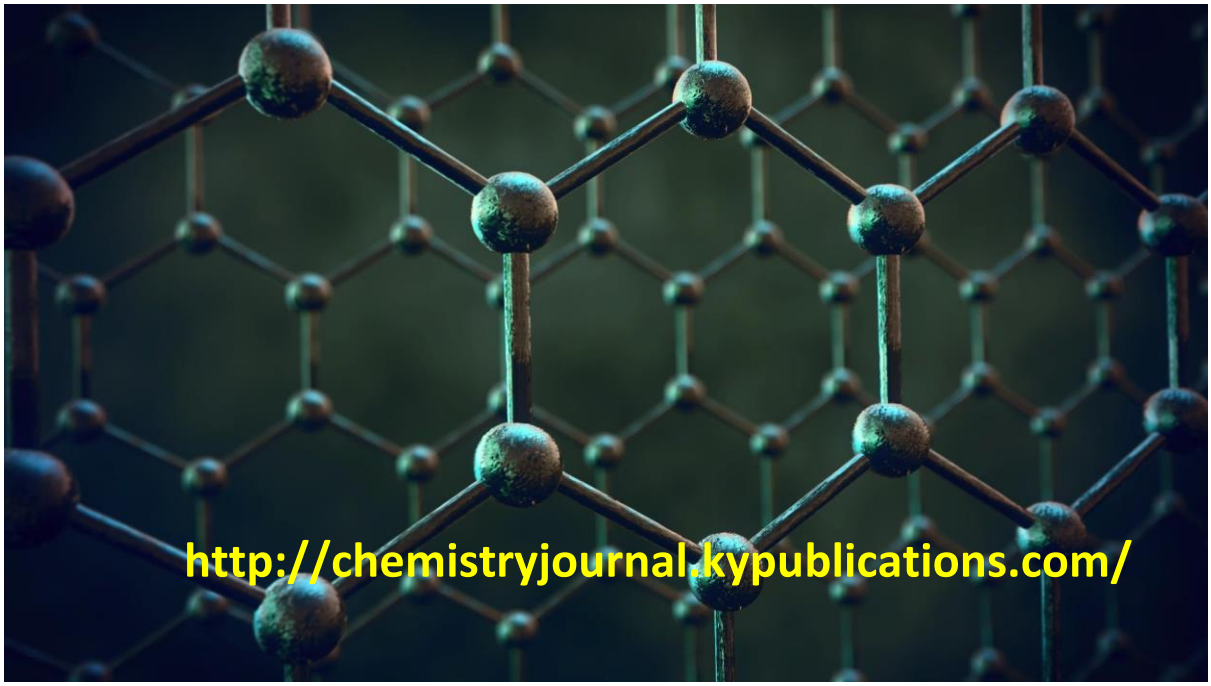

International Journal of Chemistry and Aquatic Sciences (IJCA)

http://www.chemistryjournal.kypublications.com/

ISSN: 2455-040X

Editor-in-Chief
Dr.Y.H.Rao
Email:submitijca@gmail.com

©KY PUBLICATIONS
International Journal of Chemistry and Aquatic Sciences (IJCA)
Volume: 9, Issue 4, 2023 (Oct-Dec) Page 6-18
ISSN: 2455-040X
Research Article

Forced Degradation and Stability-Indicating Assay of Atenolol Tablets by Validated HPLC Method in Accordance with Indian Pharmacopoeia

Dr. K. Aparna Seetharam
Lecturer in Chemistry
Government College for Women (A), Guntur
Andhra Pradesh, India

doi: 10.33329/ijca.9.4.6

ABSTRACT

The present study investigates the forced degradation behavior of atenolol, a widely used β1-adrenergic receptor blocker, in tablet dosage form to evaluate the specificity of an analytical method described in the *Indian Pharmacopoeia (2018)*. Atenolol samples were subjected to a range of stress conditions, including thermolysis, photolysis, hydrolysis (acidic, basic, and moisture-induced), oxidation, and exposure to metal ions. High-performance liquid chromatography (HPLC) was employed using a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase comprising sodium heptanesulfonate, dibasic sodium phosphate (pH 3.0), methanol, and dibutylamine (70:30:2 v/v/v). Detection was carried out at 226 nm using a UV-DAD detector.

Seven degradation products were identified across different stress conditions, though none overlapped with the retention time of atenolol, confirming method specificity. No degradation was observed in placebo or diluent preparations, and atenolol showed stability only under metal ion exposure. Co-validation studies demonstrated compliance with ICH Q2(R1) guidelines, confirming the method's accuracy, precision, linearity, robustness, and recovery within acceptance limits. These results establish the method as stability-indicating and suitable for routine quality control and regulatory applications in assessing atenolol tablets.

Keywords: Atenolol; Forced degradation; HPLC; Stability-indicating method; Validation; Indian Pharmacopoeia**.**

1. Introduction

The stability of pharmaceutical formulations is influenced not only by environmental conditions such as temperature, moisture, and light, but also by product-specific factors, including the physicochemical characteristics of the active pharmaceutical ingredients (APIs) and excipients, the dosage form and its composition, the method of manufacture, and the nature and quality of packaging

materials (ICH, 2003). Stability testing provides evidence of how product quality changes over time under the influence of these factors, thereby enabling the determination of shelf life, re-test period, and appropriate storage conditions.

In India, stability studies are carried out in accordance with the Indian Pharmacopoeia (2018) and guidelines issued by the Central Drugs Standard Control Organization (CDSCO), which align with the recommendations of the International Council for Harmonisation (ICH, 2003). These guidelines emphasize the quantification of degradation products and the validation of analytical methods. Drugs must be subjected to stress conditions to generate degradation products when impurities are unknown or unavailable commercially. Such testing ensures the specificity and robustness of analytical methodologies for stability studies.

Concerns regarding impurities and degradation products in drugs and excipients remain a central issue for the pharmaceutical sector. As highlighted by Singh et al. (2012), research in this field has expanded considerably, driven both by regulatory requirements and advancements in analytical instrumentation, which now enable detection of impurities at trace levels.

In forced degradation studies, the drug is exposed to stress conditions such as thermolysis, hydrolysis (acidic and basic), oxidation, photolysis, and exposure to metal ions. These conditions generate degradation products in sufficient amounts to allow the development and validation of analytical methods for quantifying both the active substance and its degradation products. Such studies are conducted not only on the active pharmaceutical ingredient, but also on finished dosage forms, placebo formulations, and diluents, to eliminate potential interferences that could be misinterpreted as impurities. The outcomes of these studies are crucial for confirming the specificity of analytical methods, as required under ICH guidelines for analytical validation.

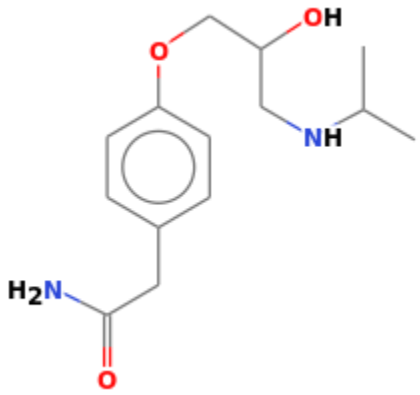

Figure 1. Molecular structure of atenolol

Atenolol, chemically known as 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzeneacetamide (Figure 1), is a selective β1-adrenergic receptor blocker. It is widely prescribed for the management of arterial hypertension, angina pectoris, cardiac arrhythmias, and as an adjuvant in the treatment of hypertrophic subaortic stenosis (Pharmacopoeia of India, 2018; Vade-Mécum, 2003).

Although atenolol is a well-established therapeutic agent, relatively few studies have focused on its stability and degradation profile. For instance, Andrisano et al. reported photostability studies in commercial formulations, while Kumar et al. investigated the role of excipients in influencing atenolol degradation. However, these studies did not encompass a comprehensive evaluation under multiple stress conditions typically recommended for forced degradation testing.

The present study, therefore, aims to investigate the forced degradation behavior of atenolol in tablet dosage form under a range of stress conditions. Additionally, it evaluates the specificity of the

analytical method prescribed in the Indian Pharmacopoeia (2018), in line with the ICH guidelines (Q1A–Q1B) on stability testing and analytical method validation, for the quantification of atenolol and its degradation products.

2. Materials And Methods

2.1 Analytical Methodology

The analytical procedure employed in this study was that described in the Indian Pharmacopoeia (2018) for atenolol tablets. The method was co-validated for parameters including system suitability, accuracy, precision, linearity, robustness, and specificity, in accordance with the requirements of ICH Q2(R1) for analytical method validation. The linearity of the method was assessed within a concentration range of 80–120%.

2.2 Chemicals and Reagents

All reagents used were of analytical or chromatographic grade, and Milli-Q® ultrapure water was employed throughout. A primary standard of atenolol with 99.6% declared purity (Indian Pharmacopoeia reference standard) was used. Placebo samples were prepared using the excipients of the formulation, which included sodium starch glycolate, magnesium stearate, sodium lauryl sulfate, magnesium carbonate, and microcrystalline cellulose.

2.3 Chromatographic Conditions

Chromatographic evaluation was carried out on a Merck-Hitachi HPLC system fitted with a UV-DAD detector and operated through Ezchrom Elite software. The analyte separation was performed using a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm), maintained at a temperature of 25 °C. The mobile phase was composed of sodium heptanesulfonate, anhydrous dibasic sodium phosphate adjusted to pH 3.0, methanol, and dibutylamine in the proportion of 70:30:2 (v/v/v). The system was run at a flow rate of 0.6 mL/min with an injection volume of 10 µL, and detection was monitored at a wavelength of 226 nm.

2.4 Equipment for Degradation Studies

Different equipment was employed depending on the stress condition. In hydrolysis studies, samples were exposed in a Mecalor Climatic Chamber (Model EC/1.2/AR-URC) under conditions of 75 ± 5% relative humidity and 40 ± 2 °C. Photolysis was carried out using a Mecalor Photostability Chamber (Model EC/0.2/R-F), in which samples were exposed to a total of 200 W·h/m² of UVA radiation. Oxidation, acid and base hydrolysis, and exposure to metal ions were performed using a Nova Water Bath (Model 314-6D), maintained at 40 °C.

2.5 Preparation of Solutions

The preparation of the standard solution involved accurately weighing 20.0 mg of atenolol reference standard into a 100 mL volumetric flask, dissolving it in the mobile phase, and further diluting 5 mL of this stock solution to 100 mL with the same solvent system. The resulting solution was mixed thoroughly and filtered through a 0.45 µm membrane filter. For the sample preparation, ten atenolol tablets (100 mg each) were placed in a 1000 mL volumetric flask with 500 mL of mobile phase, sonicated for 15 minutes, and then made up to volume with mobile phase. From this solution, a 1 mL aliquot was transferred to a 100 mL volumetric flask, diluted to volume with mobile phase, mixed, and filtered through a 0.45 µm membrane filter. The placebo preparation was carried out in an identical manner, except that an equivalent amount of placebo powder was used in place of the tablets. The mobile phase alone was injected as the diluent control.

2.6 Preparation of Degraded Samples

The degraded samples were prepared following the same procedure as the control solutions, after exposure of the tablets or solutions to stress-inducing agents. For oxidation, acid hydrolysis, base hydrolysis, and exposure to metal ions, the mobile phase was combined with hydrogen peroxide, hydrochloric acid, sodium hydroxide, or copper sulphate, respectively, and the solutions were kept in a water bath at 40 °C for two days before further dilution and filtration. For thermolysis, hydrolysis under humidity, and photolysis, atenolol tablets were exposed outside their primary packaging to a hot air oven at 60 °C, a climatic chamber at 40 °C and 75% RH, or a photostability chamber with UVA radiation, respectively, for seven days. After exposure, solutions were prepared as described for the control condition.

Table 1. Preparation of samples under different degradation conditions

ID	Degradation Condition	Stress-Inducing Agent	Exposure Conditions	Diluent Used
1	Oxidation	3% H_2O_2	2 days in water bath at 40 °C	Mobile phase with 3% H_2O_2
2	Acid Hydrolysis	1 M HCl	2 days in water bath at 40 °C	Mobile phase with 1 M HCl (pH 1.0)
3	Basic Hydrolysis	1 M NaOH	2 days in water bath at 40 °C	Mobile phase with 1 M NaOH (pH 12.0)
4	Photolysis	Ultraviolet light	7 days exposure	Mobile phase
5	Thermolysis	Elevated temperature	60 °C for 7 days	Mobile phase
6	Hydrolytic (Moisture)	75% relative humidity	7 days at 40 °C	Mobile phase
7	Metal Ion Exposure	0.05 M $CuSO_4$	2 days in water bath at 40 °C	Mobile phase with 0.05 M $CuSO_4$

3. Results & Discussion

3.1 Co-validation of the Analytical Methodology

The analytical method was evaluated for the parameters of system suitability, accuracy, precision, linearity, and robustness. The results of these validation studies are summarized in Table 2. The method was found to meet all acceptance criteria specified by ICH Q2(R1), confirming its reliability for the determination of atenolol in tablet dosage form.

3.2 Forced Degradation Study

To assess the formation of potential degradation products, atenolol samples were subjected to various stress conditions, including thermolysis, hydrolysis (acidic, basic, and moisture-induced), oxidation, photolysis, and exposure to metal ions. A total of seven degradation products were observed across these stress conditions.

The degradation products formed under each condition, along with their corresponding retention times and average peak areas in both standard and sample preparations, are presented in Table 3. In the control preparations, only the peak corresponding to atenolol was detected (Figure 2),

with no additional peaks observed. Similarly, under the condition of exposure to metal ions, no degradation product was formed, as shown in Figure 3.

Table 2. Results of co-validation parameters for the analytical method

Parameter	Acceptance Criteria	Result Obtained
System suitability	%RSD area ≤ 2.0% %RSD retention time < 2.0% Theoretical plates > 5,000 Tailing factor (asymmetry): 0.8–2.0	%RSD area: 0.439% %RSD retention time: 0.014% Theoretical plates: 11,176 Asymmetry: 1.097
Robustness	Difference between freshly prepared solution and solution after 24 h < 2.0%	0.14%
Precision (Repeatability)	%RSD < 2.0%	Day 1: 0.38% Day 2: 0.47%
Intermediate Precision (Accuracy between days)	%RSD < 4.0%	0.44%
Linearity	Correlation coefficient (r) > 0.99 F-test < 4240	r = 0.9985 F = 1.24×10^4
Accuracy	Mean recovery and individual values: 98.0–102.0%	Mean: 99.68% Minimum: 98.51% Maximum: 100.82%

Table 3. Degradation products of atenolol under different stress conditions

Stress Condition	Degradation Product	Retention Time (min) – Standard	Peak Area – Standard	Retention Time (min) – Sample	Peak Area – Sample
Oxidation	Impurity 1	—	—	0.563	224,973
	Impurity 2	—	—	6.653	14,485
	Impurity 3	—	—	7.437	2,482
	Impurity 4	8.624	1,540	8.607	13,920
Acid Hydrolysis	Impurity 1	—	—	0.687	290,907
Basic Hydrolysis	Impurity 1	—	—	0.627	315,501
	Impurity 5	4.218	8,568	3.607	5,209
	Impurity 6	6.142	3,416	6.193	1,689
Photolysis	Impurity 7	4.611	2,901	4.703	1,210
Thermolysis	Impurity 6	6.184	2,360	6.173	2,103

Moisture Hydrolysis	Impurity 6	6.180	2,169	6.167	2,651
Metal Ion Exposure	No impurities	–	–	–	–

3.3 Forced Degradation Findings

In the stress conditions of photolysis, thermolysis, and hydrolysis, the formation of a distinct degradation product was observed in each case, as illustrated in Figures 4, 5, and 6, respectively. Under oxidation, acid hydrolysis, and basic hydrolysis conditions, different degradation products were detected between the standard and sample preparations, as shown in Figures 7a/7b to 9a/9b. Importantly, no degradation was observed in the placebo and diluent preparations, nor was any peak detected at the retention time corresponding to Atenolol.

3.4 Specificity

The chromatograms obtained under all tested conditions confirmed that none of the degradation peaks overlapped with the retention time of Atenolol. Peak purity, evaluated using the DAD detector, confirmed the absence of co-eluting peaks, with Atenolol consistently showing a chromatographic purity of 1.000000 (Figure 10). These findings confirm the specificity of the method.

3.5 Validation and Stability Indication

The co-validation results (Table 2) confirm that the analytical method is stability-indicating and validated for quantifying Atenolol in tablets. The applied stress conditions in the forced degradation study were effective in generating degradation pathways, with Atenolol remaining stable only in the presence of metal ions.

3.6 Sensitivity to Environmental Factors

The degradation observed under thermolysis, photolysis, and humidity stresses highlights the need for careful evaluation of accelerated and long-term stability studies, particularly concerning storage and packaging. The findings emphasize Atenolol's sensitivity to environmental factors such as temperature, humidity, and light. However, it is noteworthy that forced degradation studies are performed under exaggerated conditions and, therefore, serve only as predictive tools. As highlighted by Andrisano et al. (1999), standard Atenolol preparations exposed to photodegradation yielded several degradation products, while the commercial formulation did not. Thus, definitive stability specifications must be established through studies conducted under conditions.

3.7 Role of Excipients in Degradation

The occurrence of distinct degradation products in sample formulations compared to the pure standard under oxidation, acid hydrolysis, and basic hydrolysis conditions suggests the involvement of excipients in degradation pathways. Specifically, impurities 1–4 (Table 3) appear to result from interactions between Atenolol and formulation excipients, absent in the isolated drug. Kumar et al. (2009) reported similar excipient–drug interactions, with decomposition of Atenolol increasing by approximately 3% or more in marketed formulations. Among the excipients studied, sodium starch glycolate and microcrystalline cellulose—both present in the tested formulation—likely contribute to the observed impurity profile, warranting further evaluation in product stability studies.

3.8 Specificity of the Analytical Method

A critical review of the degradation data, particularly the analysis of peak overlap and purity, confirms that the employed analytical method is highly specific for Atenolol.

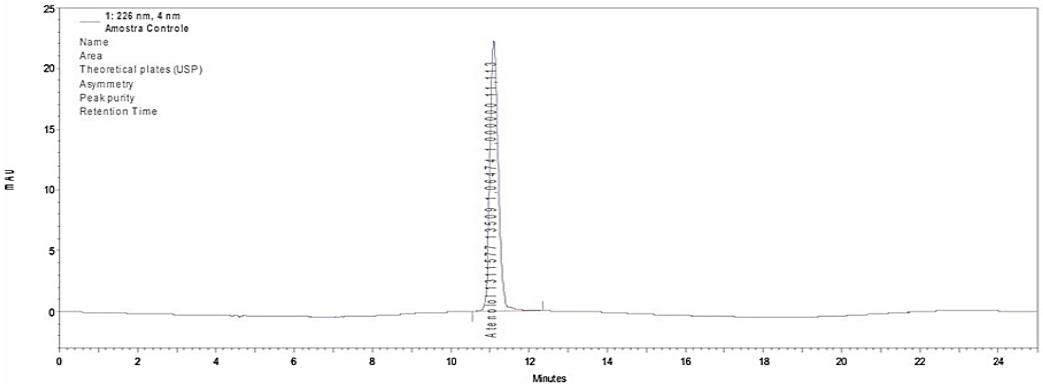

Figure 2. Chromatogram of the atenolol sample solution in the control condition

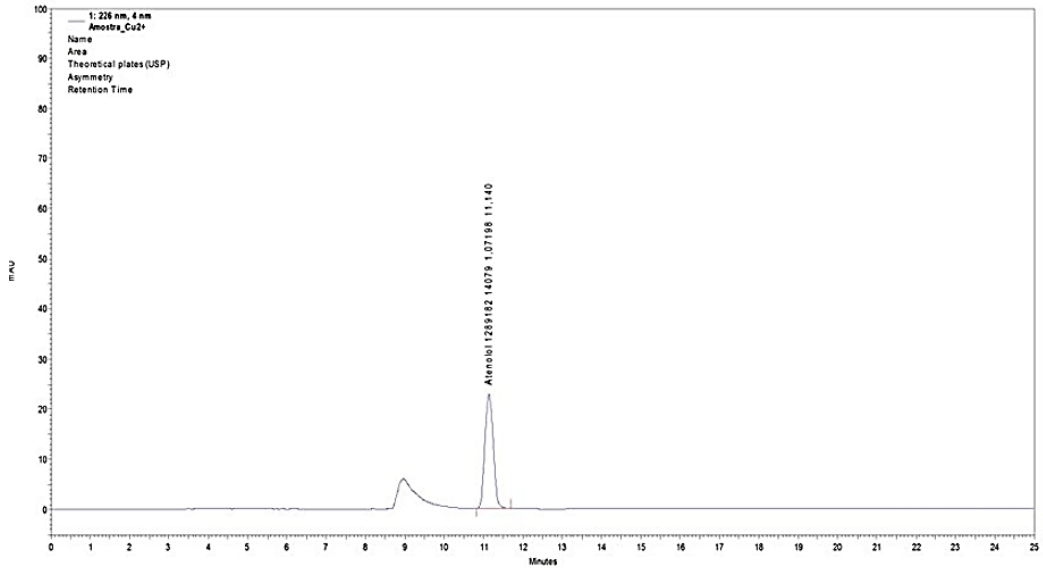

Figure 3. Chromatogram of the atenolol sample solution under the condition of exposure to metal ions

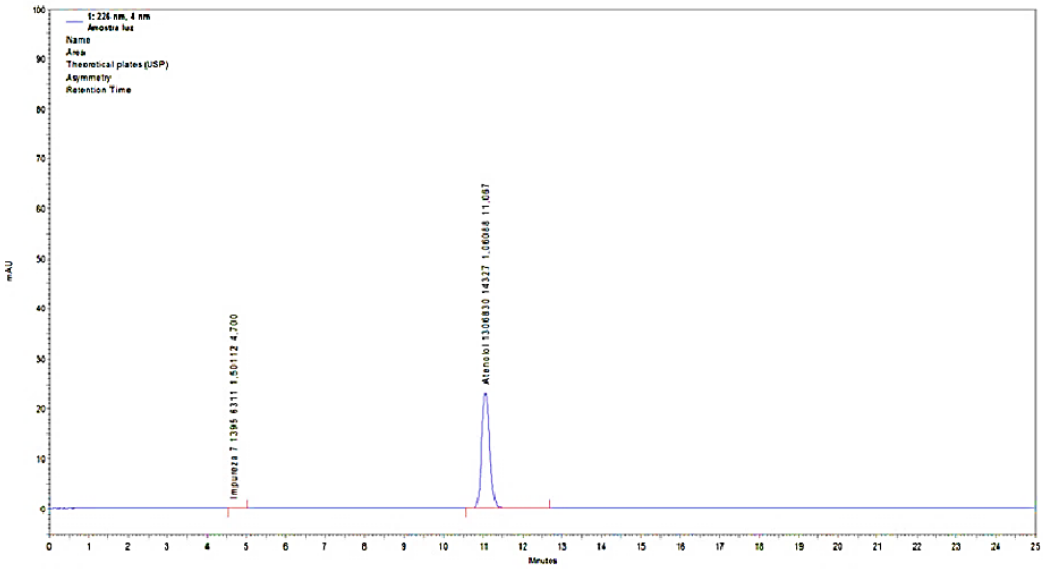

Figure 4. Chromatogram of the atenolol sample solution in the photolysis condition.

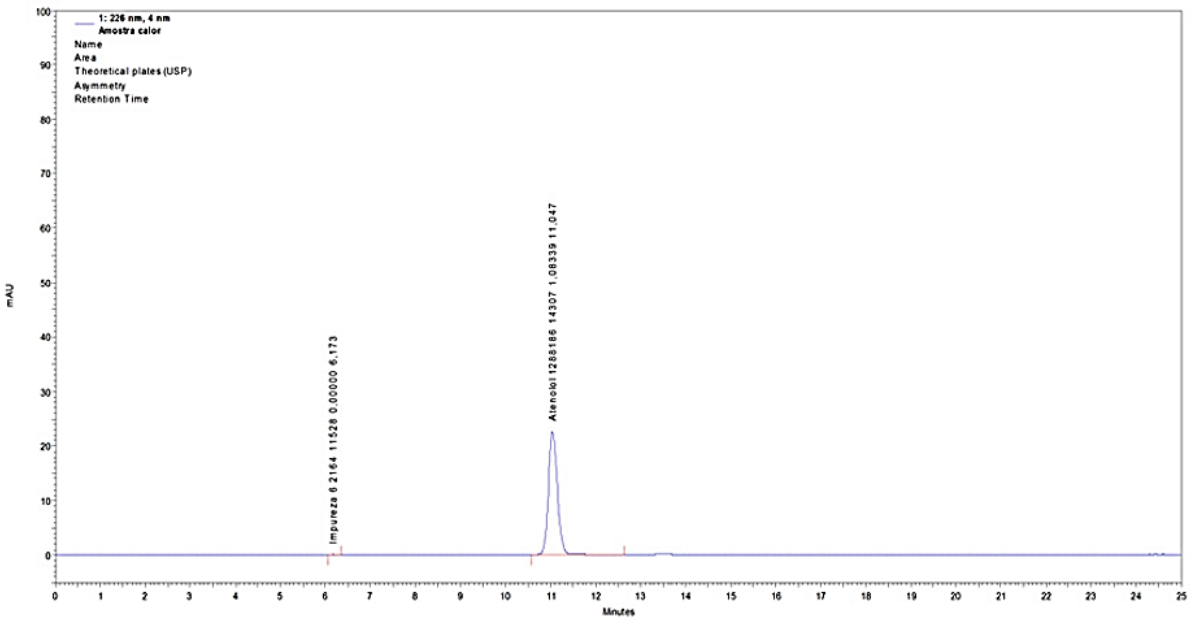

Figure 5. Chromatogram of the atenolol sample solution in thermolysis condition.

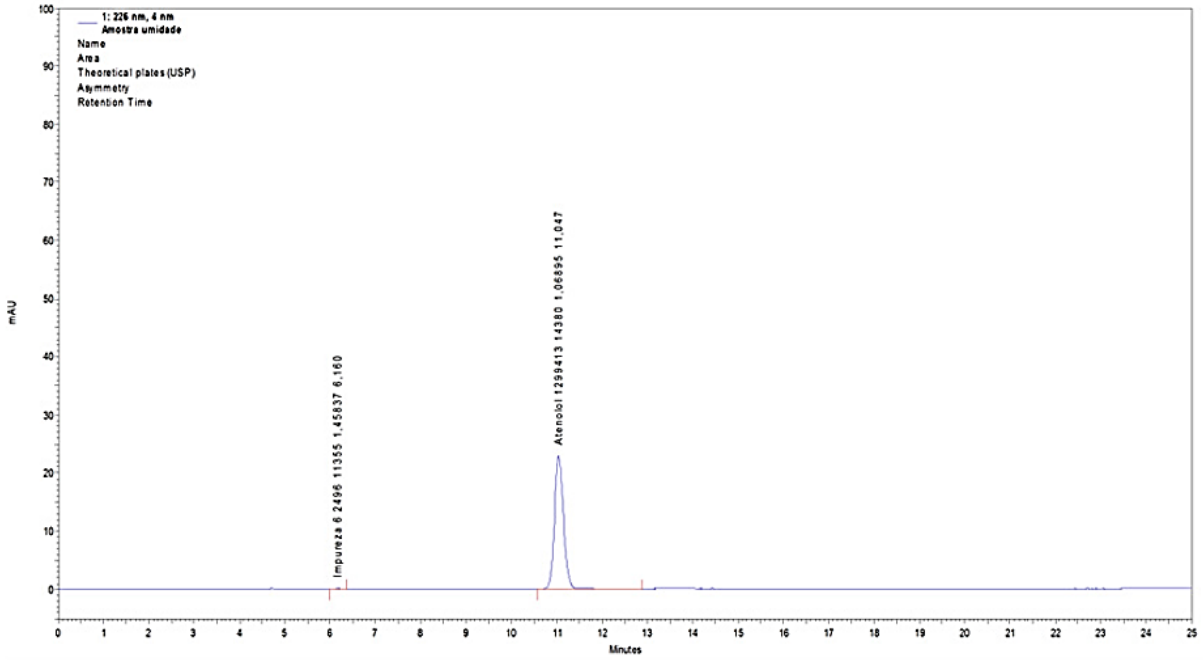

Figure 6. Chromatogram of the atenolol sample solution in hydrolysis condition.

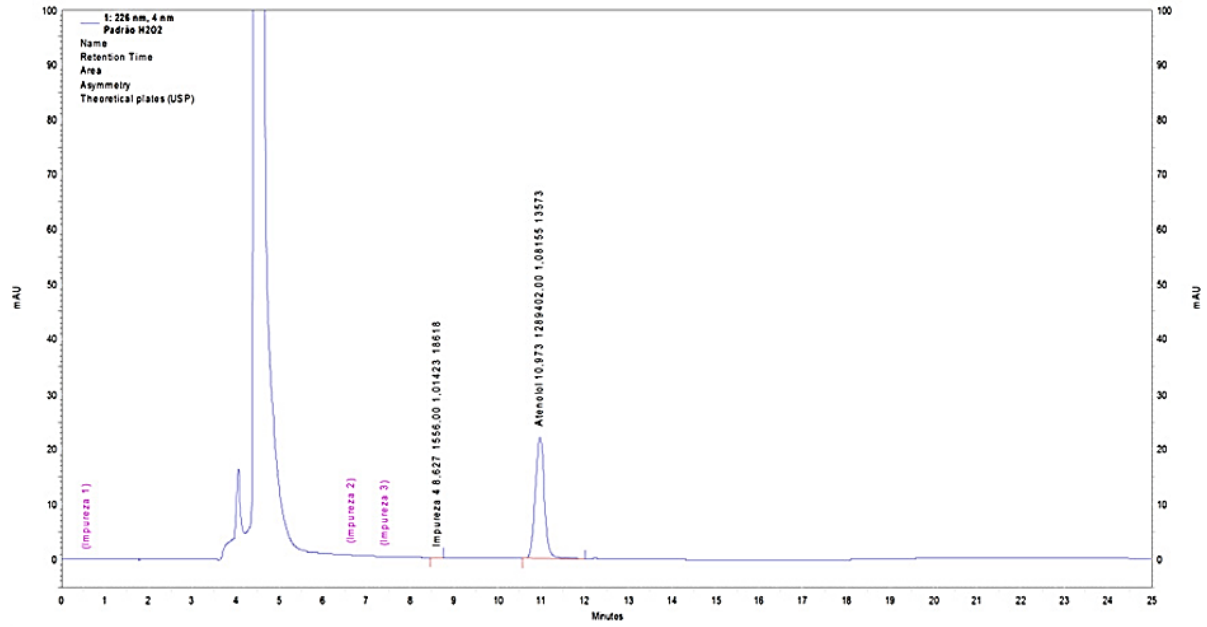

Figure 7a. Chromatogram of atenolol standard solution in oxidation condition.

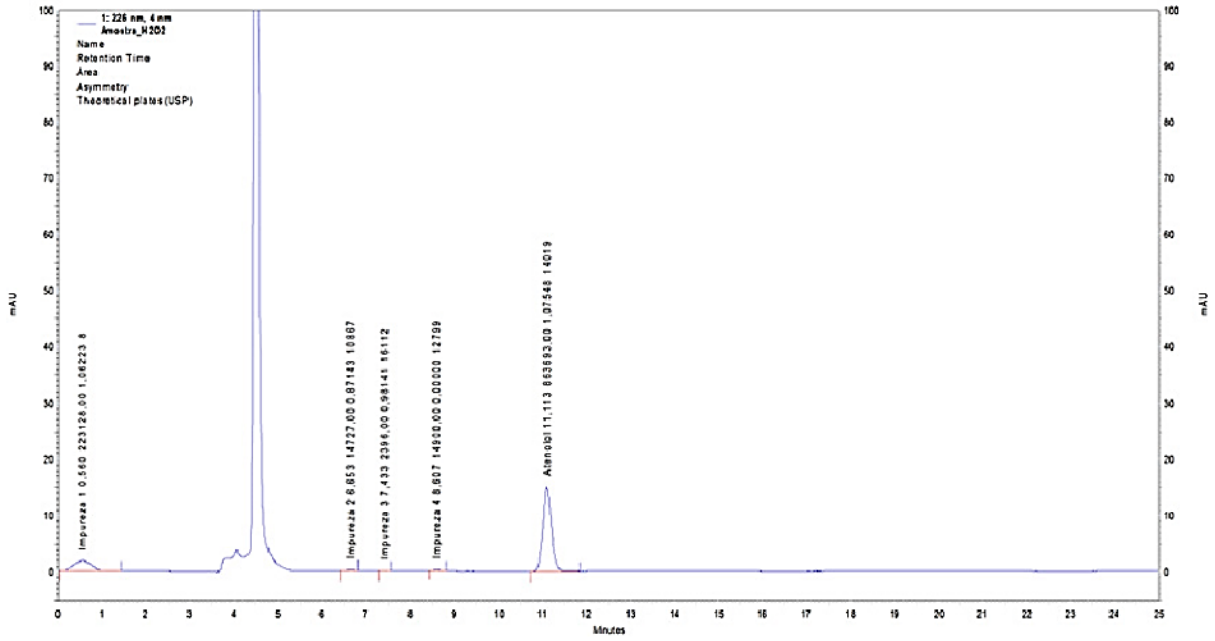

Figure 7b. Chromatogram of the atenolol sample solution in the oxidation condition.

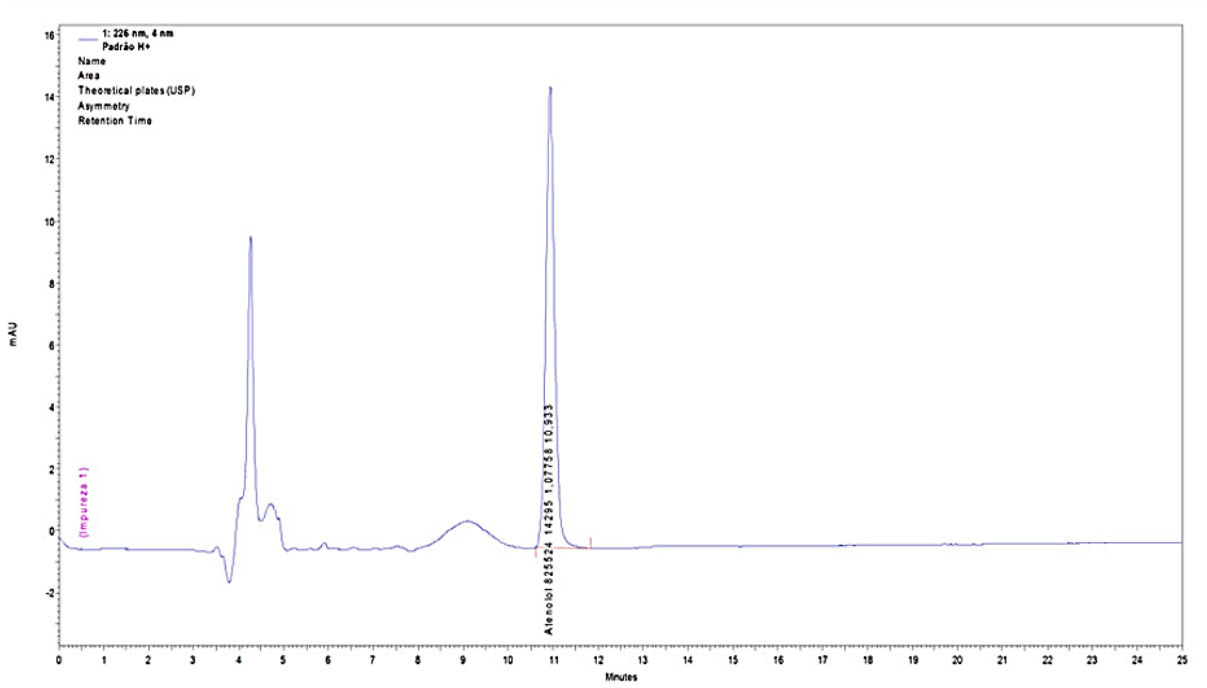

Figure 8a. Chromatogram of the standard solution of atenolol in the Acid hydrolysis condition.

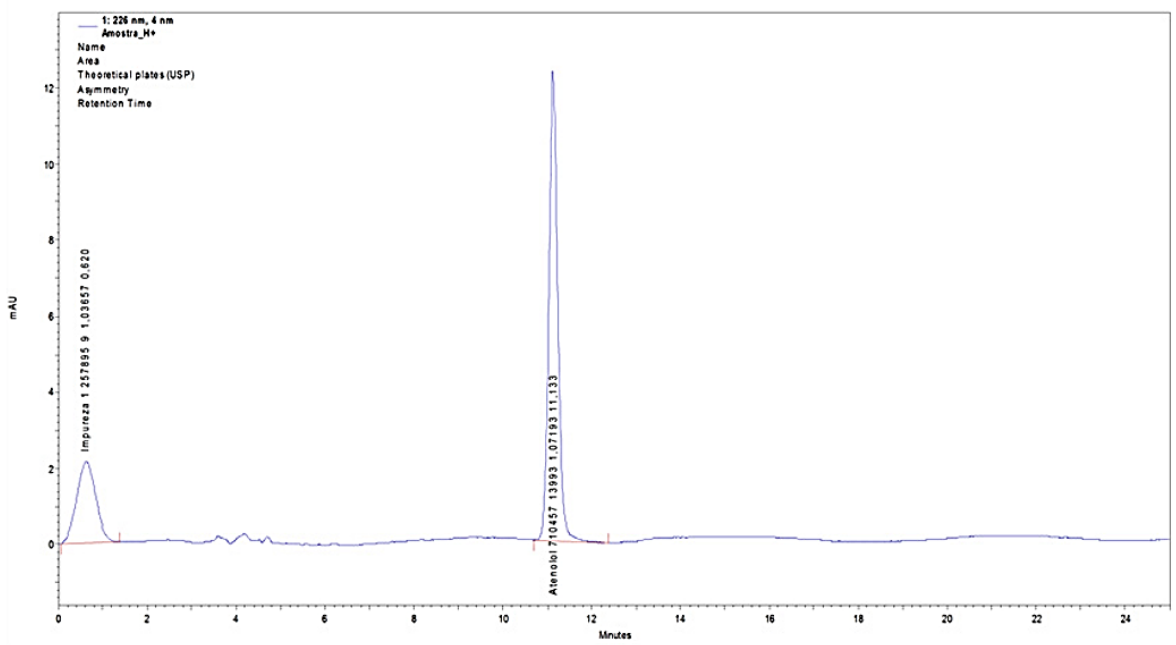

Figure 8b. Chromatogram of the atenolol sample solution in the Acid hydrolysis condition

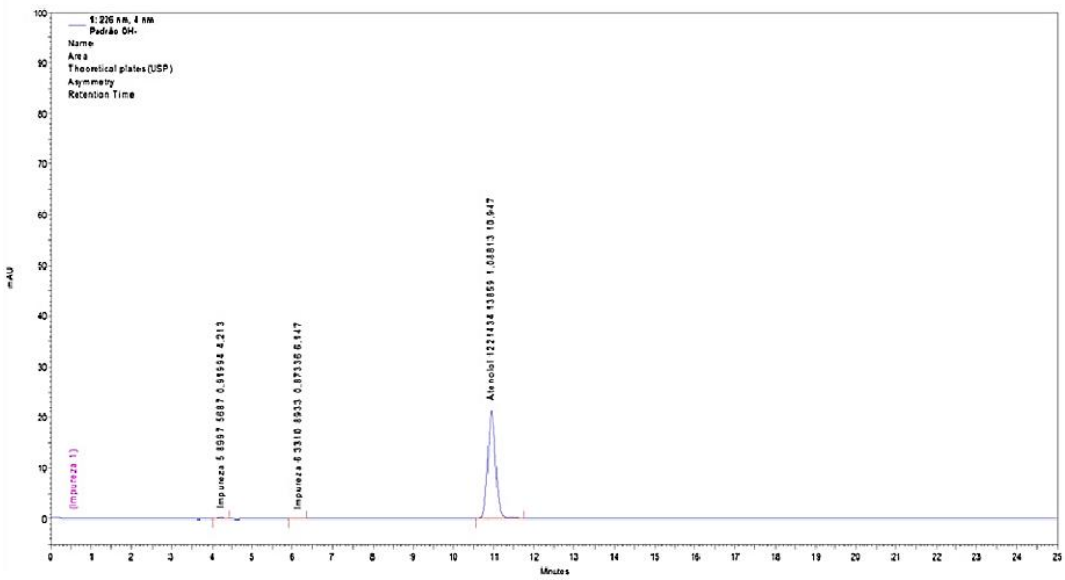

Figure 9a. Chromatogram of the standard solution of atenolol in the basic hydrolysis condition.

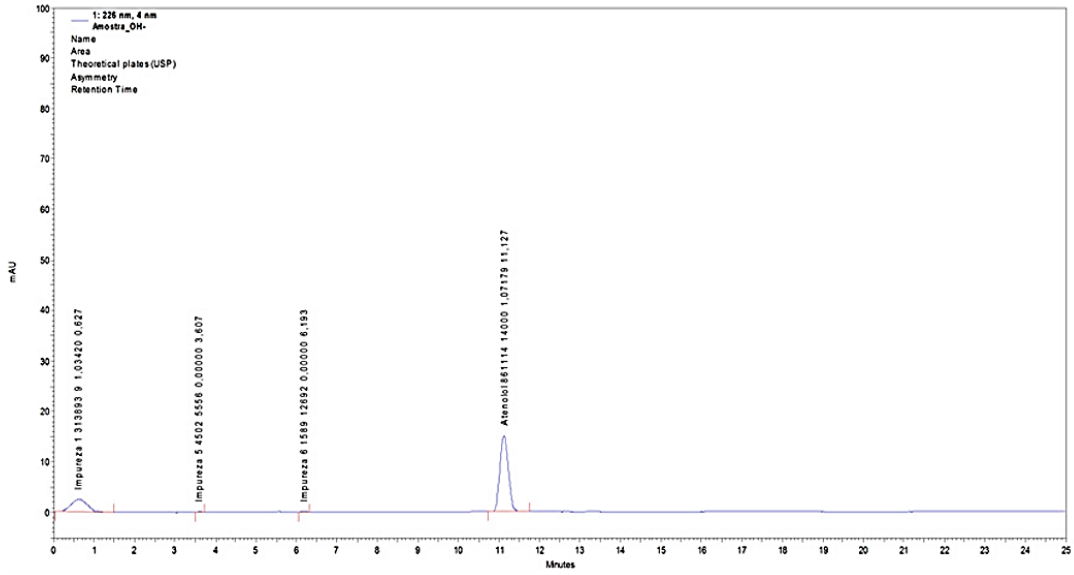

Figure 9b. Chromatogram of the atenolol sample solution in the basic hydrolysis condition.

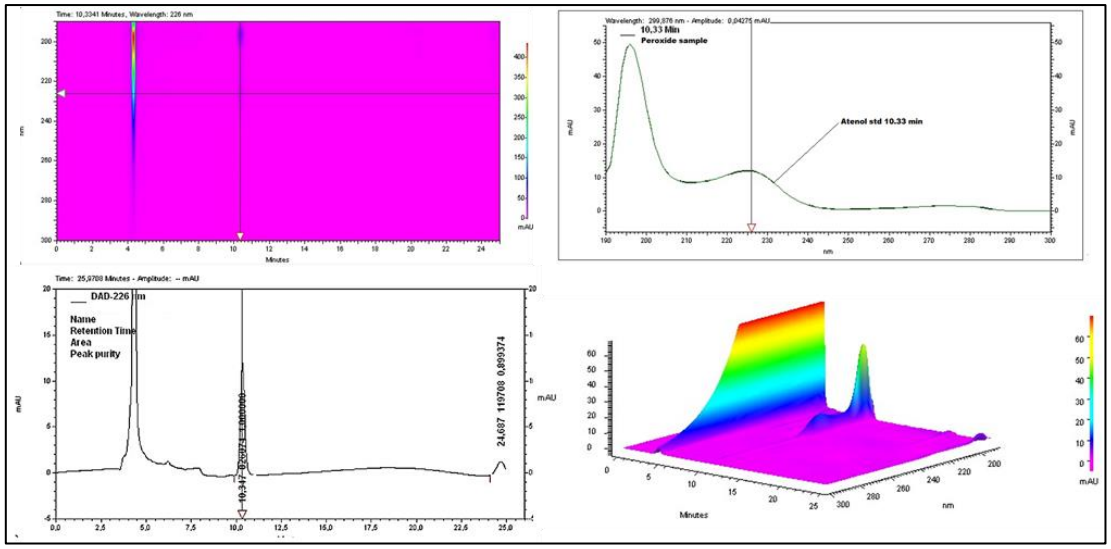

Figure 10. Chromatographic purity of atenolol sample solution in oxidation condition.

4. Conclusion

The present study successfully demonstrated the forced degradation behavior of atenolol tablets under various stress conditions and confirmed the suitability of the analytical methodology described in the *Indian Pharmacopoeia (2018)*. High-performance liquid chromatography, validated in accordance with ICH Q2(R1) guidelines, proved to be robust, precise, accurate, and stability-indicating. Seven degradation products were identified across different stress conditions, with no overlap or interference at the retention time of atenolol, confirming the specificity of the method. Among the stress factors tested, atenolol showed marked susceptibility to acid and base hydrolysis, oxidation, photolysis, and exposure to temperature and humidity, while it remained stable in the presence of metal ions.

The differences in degradation profiles between standard and sample preparations highlight the significant role of excipients in influencing drug stability. In particular, the presence of sodium starch glycolate and microcrystalline cellulose in the formulation may contribute to the formation of additional impurities, warranting careful consideration in stability and compatibility studies. These findings emphasize the need for comprehensive evaluation of storage conditions, packaging materials, and formulation components during product development and regulatory approval processes.

Overall, the results confirm that the applied HPLC method is specific, validated, and suitable for routine quality control, degradation profiling, and stability studies of atenolol tablets. Furthermore, this research provides predictive insights into the drug's stability profile, which is crucial for ensuring therapeutic efficacy, patient safety, and regulatory compliance. Future studies under real-time and accelerated stability conditions will complement these findings and support definitive shelf-life determinations.

References

[1]. Andrisano, V., Gotti, R., Di Pietra, A. M., Cavrini, V., & Andrisano, G. (1999). Photostability studies on atenolol in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis, 21*(4), 835–844. https://doi.org/10.1016/S0731-7085(99)00173-2

[2]. International Council for Harmonisation (ICH). (2003). *Stability testing of new drug substances and products Q1A(R2)*. ICH Harmonised Tripartite Guideline. Retrieved from https://www.ich.org

[3]. International Council for Harmonisation (ICH). (2003). *Stability testing: Photostability testing of new drug substances and products Q1B*. ICH Harmonised Tripartite Guideline. Retrieved from https://www.ich.org

[4]. International Council for Harmonisation (ICH). (2005). *Validation of analytical procedures: Text and methodology Q2(R1)*. ICH Harmonised Tripartite Guideline. Retrieved from https://www.ich.org

[5]. Kumar, S., Shukla, S., & Sharma, V. (2009). Influence of excipients on the stability of atenolol in solid dosage forms. *International Journal of Pharmaceutics, 372*(1–2), 22–26. https://doi.org/10.1016/j.ijpharm.2009.01.033

[6]. Pharmacopoeia Commission for Indian Medicine & Homoeopathy. (2018). *Indian Pharmacopoeia 2018*. Ghaziabad: Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India.

[7]. Singh, S., Bakshi, M., & Chaturvedi, S. C. (2012). Pharmaceutical impurities: Regulatory aspects and analytical characterization. *Trends in Analytical Chemistry, 32*, 48–60. https://doi.org/10.1016/j.trac.2011.08.007

[8]. Vade-Mecum. (2003). P.R. Vade-Mecum: Clinical drug reference. São Paulo: Editora Artes Médicas.