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STABILITY-INDICATING METHOD FOR ASSAY AND IMPUTIRTY PROFILING FROM AMLODIPINE AND METOPROLOL IN THEIR PHARMACEUTICAL DOSAGE FORMS USING RP-HPLC WITH UV/PDA DETECTOR

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ABSTRACT

An objective of this research is to develop a stability-indicating method for the simultaneous determination of assay and impurities profiling from amlodipine and metoprolol in their pharmaceutical dosage form using reverse phase high-performance liquid chromatography (RP-HPLC) with ultraviolet-photodiode array detector. The chromatographic separation of amlodipine, metoprolol, and their impurity peaks was achieved using an YMC-Triart PFP C18 column (250 × 4.6 mm, 5 µm particle size). A mobile phase composed of buffer solution 2 and acetonitrile (60: 40 v/v) was selected, and a flow rate of 1.2 mL/minute was monitored with an injection volume of 5 µL. The column oven and autosampler temperatures were maintained at 25, and detection was performed at 235 nm. The method was validated according to the International Council on Harmonization guidelines. The method was linear and accurate as correlation coefficient and % recovery were within the acceptance criteria. System suitability criteria were also fulfilled. This method is specifically developed as a stability-indicating method to rapidly and simultaneously estimate amlodipine, metoprolol, and their impurities. All the impurities and drug substances eluted before 25 min. Hence, the run time was set as 30 and 40 min for the assay and related substance test, respectively. Because all the impurities of amlodipine eluted within 25 min, the developed RP-HPLC method is simple, fast, and economical. The projected method can be utilized for routine analysis in the quality control department of the pharmaceutical industry.

KEYWORDS: Amlodipine, Metoprolol, Stability-indicating method, Reverse phase high-performance liquid chromatography, Assay, Related Substances.

INTRODUCTION

Amlodipine besylate, chemically known as (RS)-3-ethyl-5-methyl-l-2-(2-amino-ethoxymethyl) -4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzene sulfonate, is a long-acting calcium channel blocker, which is utilized as an antihypertensive operator.[1] It is also used to treat tall blood weight (hypertension) or chest torment (angina) and other conditions caused by coronary course malady.[2] Metoprolol succinate, chemically known as (RS)-1-(Isopropylamino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol, is a specific β1-receptor blocker that is additionally utilized as an antihypertensive operator.[1] Metoprolol succinate acts as a beta-adrenergic blocking operator, diminishing chest torment and reducing tall blood weight.[3]

Within the settled dosage combination of amlodipine (calcium channel blocker) and metoprolol (cardio-selective beta-blocker); both the drugs have two distinctive components and decrease blood weight by acting on fringe vascular resistance, stroke volume, and heart rate. This combination treatment successfully accomplishes target blood weight, lowers rate of side effects of the drug, produces synergistic impacts, and expands persistent compliance.[4] For simultaneous dedication of associated materials of amlodipine and metoprolol has also been mentioned. However, no method for the simultaneous evaluation of amlodipine and metoprolol in addition to impurities in these drugs has been mentioned to date. Experiments were performed for the quantitative deduction of capsules in conjunction with their impurities using reverse phase high-performance liquid chromatography (RP-HPLC). Furthermore, metoprolol-associated compound D elutes at a retention time of approximately 55 min with respect to the associated material method from USP monographs of metoprolol tablets. However, metoprolol-associated compound D elutes at a retention time of approximately 3.5 min with the newly developed RP-HPLC method. Therefore, the modern techniques are profitable for improving and validating a unique and fast RP-HPLC method for the simultaneous estimation of amlodipine and metoprolol along with their impurities in a pharmaceutical dosage form. In addition, the price required for the RP-HPLC method could be much lower than that of the RP-UPLC method. The ICH guideline states that stress testing is performed to identify the possible degradation products, further facilitating in determining the intrinsic stability of the molecules, establishing degradation

pathways, and validating the stability-indicating procedures used.[5] To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis, or photolysis of the drug substances and drug products.[5,6] The design of the photolysis studies is left to the discretion of the applicant although Q1B specifies that the light source should produce combined visible and ultraviolet (UV, 320–400 nm) outputs, and that the exposure levels should be justified.[7] Some scientists have identified it practical to begin under extreme conditions such as 80 °C or even higher temperatures and shorter multiple-time points (such as 2, 5, 8, and 24 h) such that the degradation rate can be evaluated.[8]

MATERIALS AND METHODS

Methods development

Various mobile phase compositions and buffer systems were tried for achieving the optimum separation of amlodipine, metoprolol, and their impurities. A mobile phase composed of buffer solution 2 and acetonitrile (60: 40 v/v) afforded the optimum resolution, which was selected at a flow rate of 1.2 mL/min for further analysis. Different buffer systems were also tried to achieve suitable peak shapes and resolution. A YMC-Triart PFP C18 Column (250 mm x 4.6 mm, 5 µm particle size) was selected. To analyze drugs along with their impurities, the detection was conducted at various wavelengths; a wavelength of 235 nm was optimized for detection because both the drugs showed maximum absorption at this wavelength. The retention time was 9.21 and 21.07 min for amlodipine and metoprolol, respectively. The optimized chromatographic conditions and system suitability parameters are illustrated in Table 1.[9]

Table 1: Optimized chromatographic conditions and system suitability parameters.

Chromatographic Conditions	
Column	YMC-Triart PFP, C18 Column (250 mm x 4.6 mm, 5 µm particle size)
Flow rate	1.2 mL/minute
Injection volume	5 µL
Wavelength	235 nm
Column oven temperature	25 ℃
Autosampler temperature	25 ℃
Run time	30 and 40 min for assay and related substances
Retention time	21.07 min: amlodipine 09.20 min: metoprolol 16.75 min: amlodipine related compound A 07.91 min: metoprolol related compound A 04.93 min: metoprolol related compound B 06.94 min: metoprolol related compound C

	03.57 min: metoprolol related compound D	
System suitability parameters		
	Amlodipine	Metoprolol
Tailing factor (Limit: NMT 2.0)	1.13	1.15
Theoretical plates (Limit: NLT 2000)	12839	11992
% Relative standard deviation (RSD)	0.6	0.6

Instrumentation

According to the literature, unique assay methods such as HPTLC,[10] synchronous fluorescence spectrofluorometric method,[11] LC-MS/MS-method,[12-14] UHPLC-MS/MS,[12] HPLC,[15] RP-UPLC Method[3] for simultaneous dedication of assay of Amlodipine and Metoprolol. Moreover, the RP-UPLC[16] and HPLC analyses were performed using a Dionex Ultimate 3000 HPLC system equipped with a UV detector.[9] Chromatographic data was acquired using the Chromeleon 7.2 software.

Preparation of solutions

Buffer solution 1: sodium dihydrogen phosphate monohydrate (7.0 g) was weighed and dissolved in water (1 L). The solution pH was adjusted to 3.0 ± 0.05 using dilute orthophosphoric acid. The mixture was then filtered through 0.45-µm nylon membrane filter and mixed well.

Buffer solution 2: 1-decane sulfonic acid (1.0 g) was weighed and dissolved in water (1 L). The pH was adjusted to 3.0 ± 0.05 using dilute orthophosphoric acid. This mixture was then filtered through 0.45-µm nylon membrane filter and mixed well.

Mobile phase: buffer solution 2 and acetonitrile were mixed in the ratio of 60:40 v/v. The mobile phase was then mixed well and degassed.

Diluent: buffer solution 1 and acetonitrile were mixed in the ratio of 50:50 v/v and mixed well.

Preparation of reference solutions

Reference solution for assay: working standards were prepared by weighing approximately 27.7 and 190.5 mg of amlodipine besylate and metoprolol succinat, respectively, which were then transferred separately into a 50 mL volumetric flask. Next, 30 mL of the diluent was

added, and the resulting solution was sonicated until dissolution. The volume was made up to the mark with the diluent, and the solution was mixed well and injected (Concentration: 554 ppm of amlodipine besylate equivalent to 400 ppm of amlodipine and 3810 ppm of metoprolol succinate equivalent to 4000 ppm of metoprolol tartrate).

Reference solution for unspecified impurities: reference solution (2 mL) for assay was diluted up to 100 mL with the diluent. Next, 1.0 mL of this solution was further diluted to 10 mL with the diluent. The solution was then mixed well and injected. (Concentration: 0.8 ppm of amlodipine and 8 ppm of metoprolol tartrate).

Reference stock solution for amlodipine-related compound A: amlodipine-related compound A (2.5 mg), an impurity standard, was weighed and transferred into a 50 mL volumetric flask. Subsequently, 30 mL of the diluent was added, and the resulting solution was sonicated until dissolution. The volume was made up to the mark with the diluent, and solution was mixed well. (Concentration: 50 ppm of amlodipine-related compound A)

Reference stock solution for metoprolol-related compound A: metoprolol-related compound A (5.0 mg), an impurity standard, was weighed and transferred into a 25 mL volumetric flask. Subsequently, 15 mL of the diluent was added, and the resulting solution was sonicated until dissolution. The volume was made up to the mark with the diluent, and the solution was mixed well. (Concentration: 50 ppm of metoprolol-related compound A).

Reference stock solution for metoprolol-related compound B: metoprolol-related compound B (5 mg), an impurity standard, was weighed and transferred into a 25 mL volumetric flask. Subsequently, 15 mL of the diluent was added, and the resulting solution was sonicated until dissolution. The volume was made up to the mark with diluent, and the solution was mixed well. (Concentration: 50 ppm of metoprolol-related compound B).

Reference stock solution for metoprolol-related compound C: metoprolol-related compound C (5.) mg), an impurity standard, was weighed and transferred into a 25 mL volumetric flask. Subsequently, 15 mL of the diluent was added, and the resulting solution was sonicated until dissolution. The volume was made up to the mark with the diluent, and the solution was mixed well. (Concentration: 50 ppm of metoprolol-related compound C).

Reference stock solution for metoprolol-related compound D: metoprolol-related compound D (5.0 mg), an impurity standard, was weighed and transferred into a 25 mL volumetric flask.

Next, 15 mL of the diluent was added, and the resulting solution was sonicated until dissolution. The volume was made up to the mark with the diluent, and the solution was mixed well. (Concentration: 50 ppm of metoprolol-related compound D).

Impurity reference solution for amlodipine-related compound A and metoprolol-related compound A, B, C, and D: reference stock solutions (10 mL) for amlodipine-related compound A and metoprolol-related compound A, B, C, and D (50 ppm each) were further diluted to 25 mL with the diluent. The solutions were was mixed well and injected. (Concentration: 2 ppm each of amlodipine-related compound A and metoprolol-related Compound A, B, C, and D).

Preparation of test solution

Twenty tablets were weighed, and the average weight was calculated. These tablets were crushed to a fine powder by suitable means and transferred the crushed powder equivalent to the 20 mg of amlodipine/200 mg of metoprolol tartrate into a 50 mL volumetric flask. Next, 30 mL of the diluent was added, and the solution was sonicated for 15 min with intermittent shaking. The solution was cooled, diluted up to the mark with the diluent, mixed well, and then injected. (Concentration: 400 ppm of amlodipine and 4000 ppm of metoprolol tartrate).

Method validation and forced degradation study

The above developed and validated HPLC method is selective, linear, precise, accurate, and robust for the simultaneous estimation of amlodipine and metoprolol with their impurities in pharmaceutical dosage form in accordance with the ICH guidelines.[17] The detailed parameter of the method validation is provided in our previous study(©2021 JETIR October 2021, Volume 8, Issue 10 (ISSN-2349-5162)).

Stress degradation studies were carried out via solution and solid-state analysis.

Preparation of samples

Preparation of untreated samples: the test solution was prepared by following the procedure that is identical to that in the method development.

Preparation of treated samples

1) Acid, base, and oxidation degradation test: Twenty tablets were weighed and crushed to a fine powder by suitable means and transferred the crushed powder equivalent to the 20 mg of amlodipine/200 mg of metoprolol tartrate into three different 50 mL volumetric flask. Next,

30 mL of the diluent was added into each flask, and the solution was sonicated for 15 min with intermittent shaking. In each flask, 5 mL of 2 N HCl, 2 mL 2 N NaOH, and 5 mL of 30% H_2O_2 were added and kept at 60 °C in a water bath for 120 min. The solution was cooled and diluted up to the mark with the diluent. The solution was mixed well and centrifuged at 4000 rpm for 5 min, and the supernatant solution was injected. (Concentration: 400 ppm of amlodipine and 4000 ppm of metoprolol tartrate). The blank and placebo (weighed placebo powder equivalent to the 20 mg of amlodipine) solutions were prepared in a similar manner.

2) Thermal degradation test: Twenty tablets were weighed and crushed to a fine powder by suitable means and transferred the crushed powder equivalent to the 20 mg of amlodipine/200 mg of metoprolol tartrate into 50 mL volumetric flask. Subsequently, 30 mL of the diluent was added into the flask, and the solution was sonicated for 15 min with intermittent shaking and heated at 70 °C in a water bath for 120 min. The solution was cooled and diluted up to the mark with the diluent. The solution was mixed well and centrifuged at 4000 rpm for 5 min, and the supernatant solution was injected. (Concentration: 400 ppm of amlodipine and 4000 ppm of metoprolol tartrate). The blank and placebo (weighed placebo powder equivalent to the 20 mg of amlodipine) solutions were prepared in a similar manner.

3) Photolytic degradation test: Twenty tablets were weighed and crushed to a fine powder by suitable means. The sample was placed in a Petridish, kept in a photostability chamber, and exposed at 1.2 million lux hours. Then, the treated powder equivalent to the 20 mg of amlodipine/200 mg of metoprolol tartrate was transferred into a 50 mL volumetric flask. Next, 30 mL of the diluent was added into the flask, and the solution was sonicated for 15 min with intermittent shaking. The solution was cooled and diluted up to the mark with the diluent. The solution was then mixed well and centrifuged at 4000 rpm for 5 min, and the supernatant solution was injected. (Concentration: 400 ppm of amlodipine and 4000 ppm of metoprolol tartrate). The blank and placebo (weighed placebo powder equivalent to the 20 mg of amlodipine) solutions were prepared in a similar manner.

Specificity

Spectral purities of the chromatographic peaks of amlodipine, metoprolol, and their impurities were evaluated for the interference of the diluent and placebo. The chromatographic peaks should be well separated, and there should be no co-elution of impurities with the main peak and other peaks. The peak purity should not be less than 950

for amlodipine, metoprolol, and their impurity peaks. For specificity, chromatograms of the blank, reference, test, placebo, and individual impurity solutions were compared.

RESULTS AND DISCUSSION

By comparing the chromatograms of the blank, reference, test, placebo, and individual impurity solutions, no interference of any peak at the retention time of amlodipine, metoprolol, and impurities was observed. The chromatographic peaks were well separated, and there was no co-elution of impurities with the main peak and other peaks. Refer Fig. 1 to 9 for the representative chromatograms of the blank, placebo, and test solutions. The mass balance was achieved for all condition, which was not less than 95.0%. Refer Table 2 for observation and results of analysis.

Table 2: Representative table for observation and results of analysis.

Sr. No.	Condition	% Assay	% RS	Mass Balance (%)
1	As such	100.64	0.219	NA
2	Acid	85.21	14.989	99.35
3	Base	94.07	4.953	98.18
4	Oxidation	84.14	15.850	99.14
5	Thermal	84.33	15.308	98.79
6	Photolytic	101.80	0.193	101.12

Representative Chromatogram of different degradation conditions are mentioned below.

Untreated sample

By comparing the chromatograms of the blank, reference, test, placebo, and individual impurity solutions, no interference of any peak at the retention time of amlodipine, metoprolol, and impurities was observed. The chromatographic peaks were well separated, and there was no co-elution of impurities with the main peak and other peaks. Refer Fig. 1 to 8 for the representative chromatograms of the blank, placebo, reference, test, and impurity solutions.

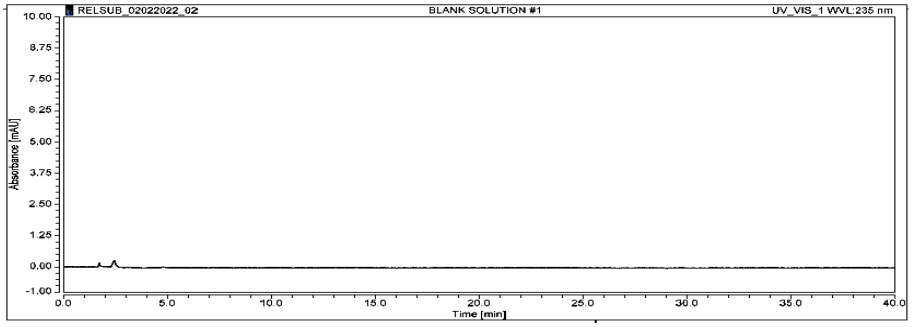

Fig. 1: Representative chromatogram of untreated blank solution.

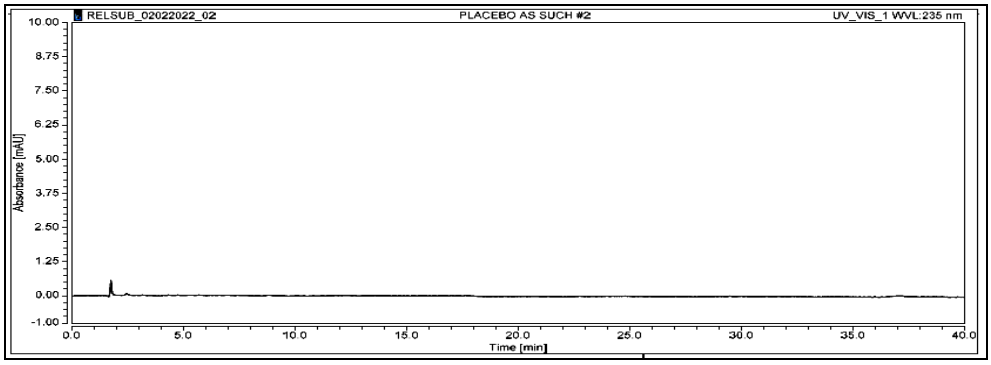

Fig. 2: Representative chromatogram of untreated placebo solution.

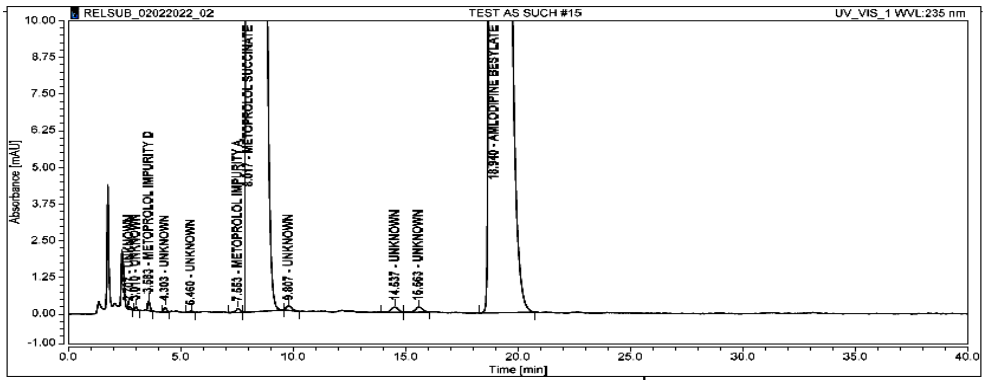

Fig. 3: Representative chromatogram of untreated test solution.

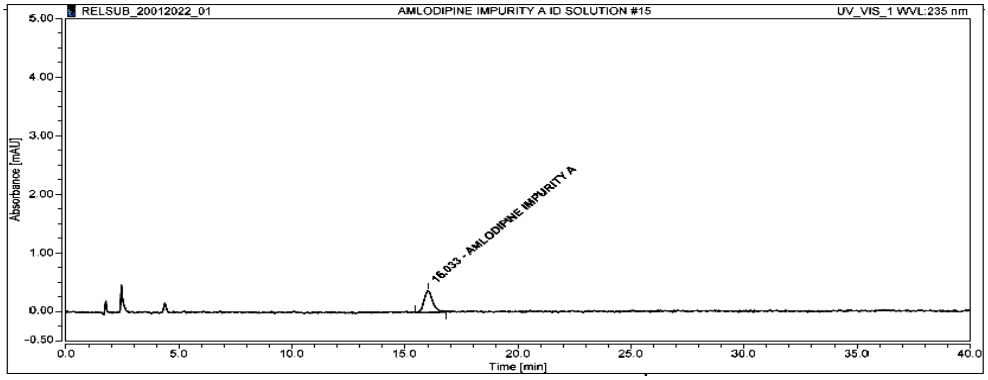

Fig. 4: Representative chromatogram of amlodipine impurity A.

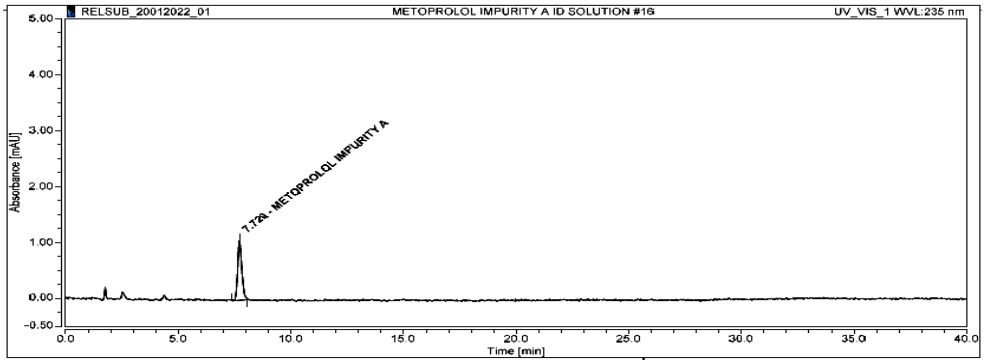

Fig. 5: Representative chromatogram of metoprolol impurity A.

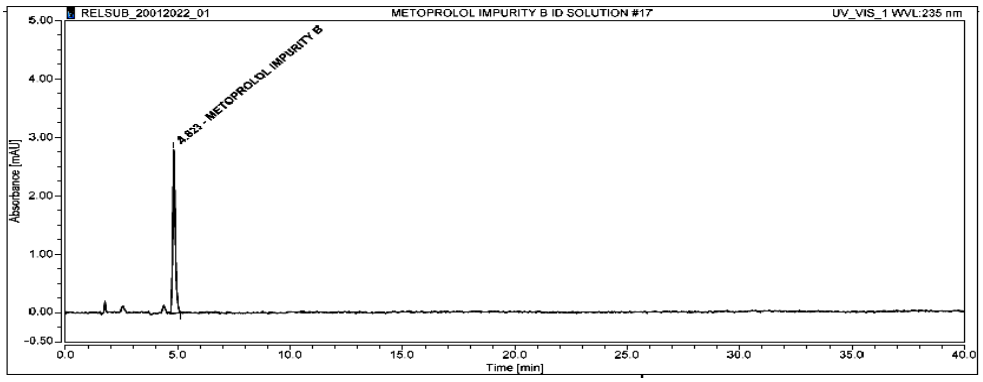

Fig. 6: Representative chromatogram of metoprolol impurity B.

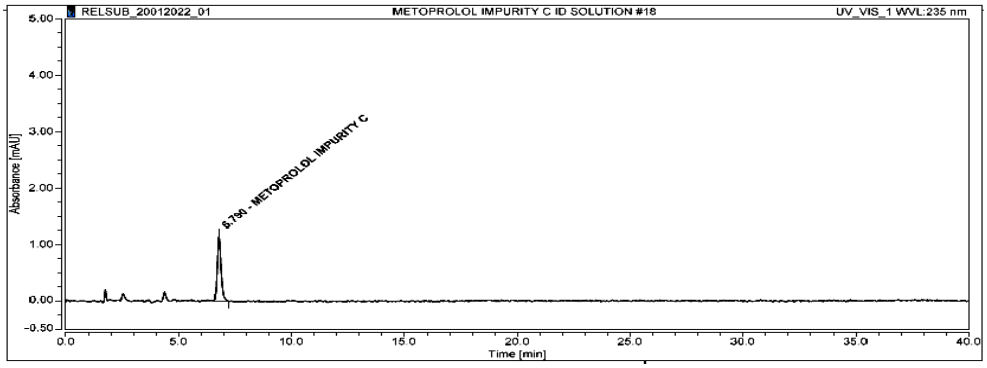

Fig. 7: Representative chromatogram of metoprolol impurity C.

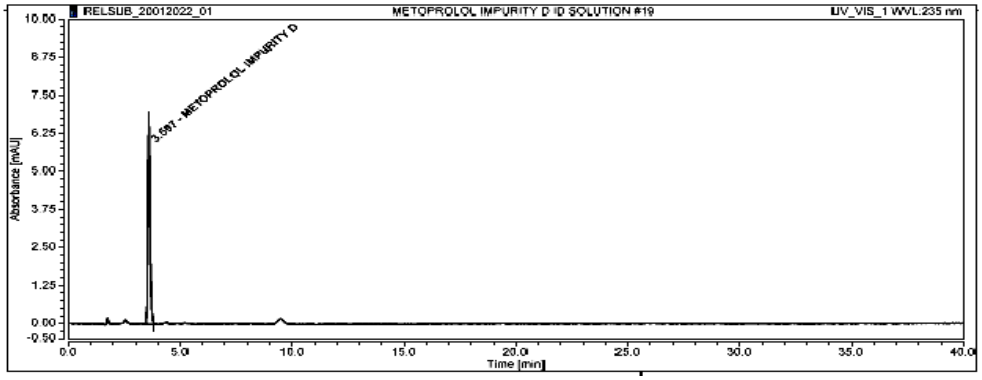

Fig. 8: Representative chromatogram of metoprolol impurity D.

Acid degradation

By comparing the chromatograms of the blank, reference, test, placebo, and individual impurity solutions, no interference of any peak at the retention time of amlodipine, metoprolol, and impurities were observed. The chromatographic peaks were well separated, and there was no co-elution of impurities with the main peak and other peaks. Refer Fig. 9 to 11 for the representative chromatograms of the blank, placebo, and test solutions.

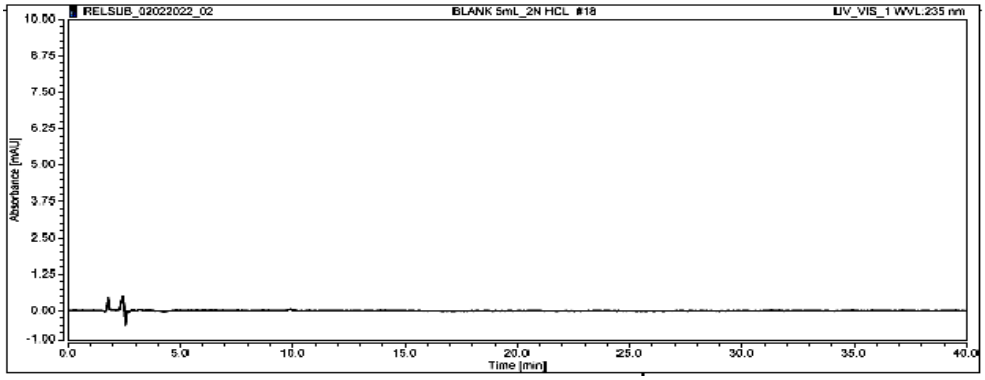

Fig. 9: Representative chromatogram of the blank solution for the acid degradation test.

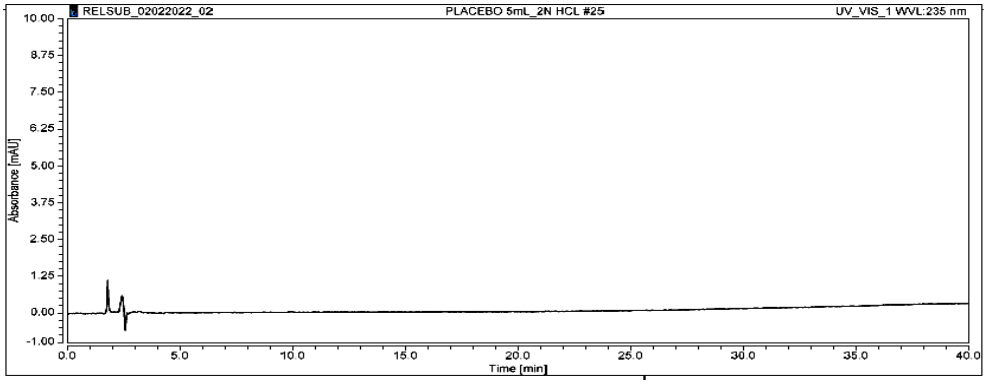

Fig. 10: Representative chromatogram of the placebo solution for the acid degradation test.

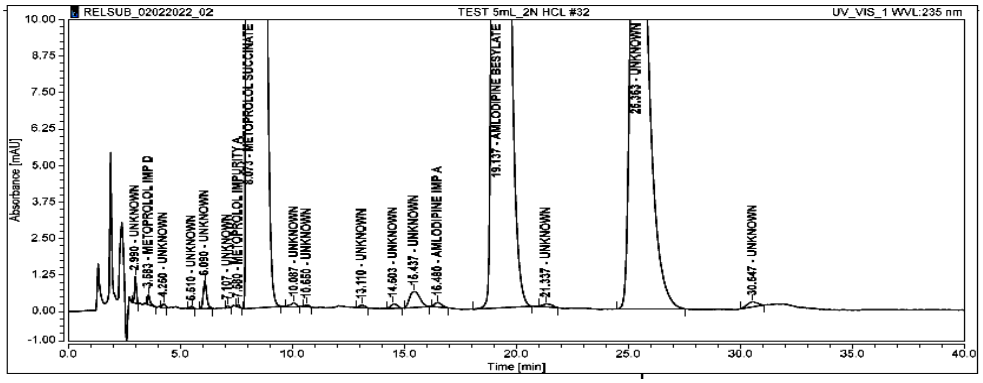

Fig. 11: Representative chromatogram of the test solution for the acid degradation test.

Base degradation

By comparing the chromatograms of the blank, reference, test, placebo, and individual impurity solutions, no interference of any peak at the retention time of amlodipine, metoprolol, and impurities was observed. The chromatographic peaks were well separated, and there was no co-elution of impurities with the main peak and other peaks. Refer Fig. 12 to 14 for the representative chromatograms of the blank, placebo, and test solutions.

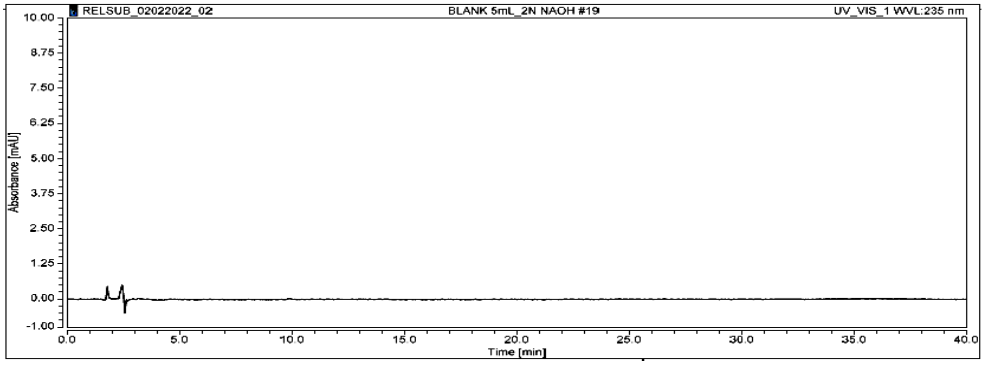

Fig. 12: Representative chromatogram of the blank solution for the base degradation test.

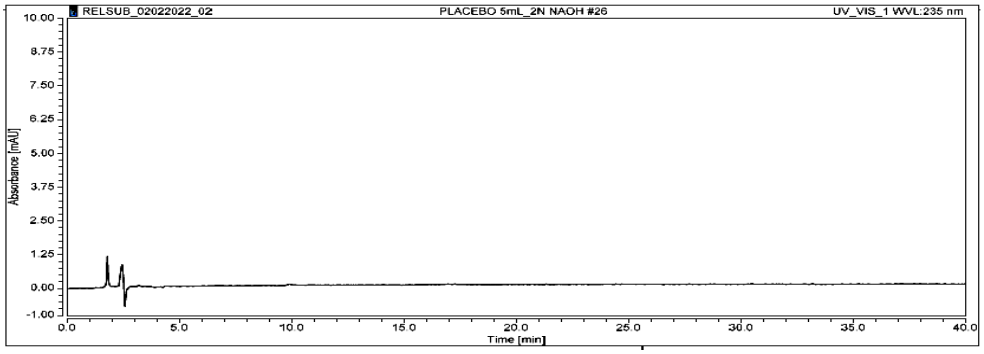

Fig. 13: Representative chromatogram of the placebo solution for the base degradation test.

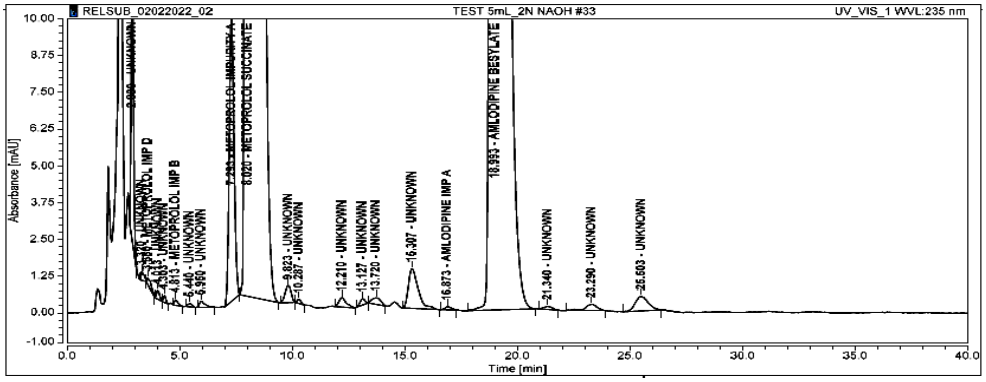

Fig. 14: Representative chromatogram of the test solution for the base degradation test.

Oxidation degradation

By comparing the chromatograms of the blank, reference, test, placebo, and individual impurity solutions, no interference of any peak at the retention time of amlodipine, metoprolol, and impurities was observed. The chromatographic peaks were well separated, and there was no co-elution of impurities with the main peak and other peaks. Refer Fig. 15 to 17 for the representative chromatograms of the blank, placebo, and test solutions.

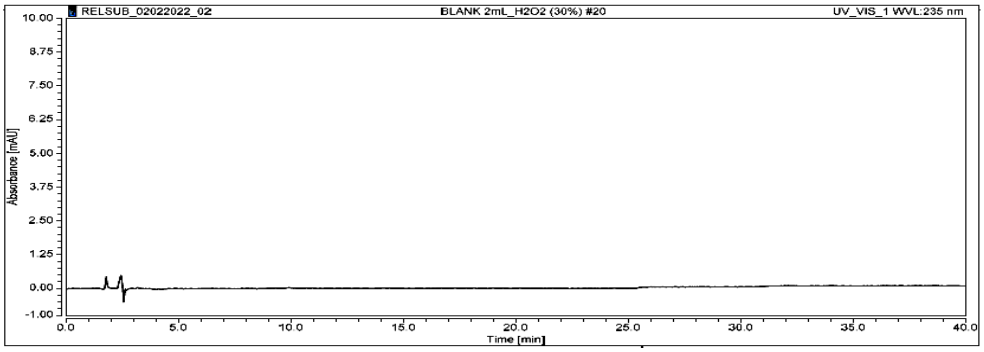

Fig. 15: Representative chromatogram of the blank solution for the oxidation degradation test.

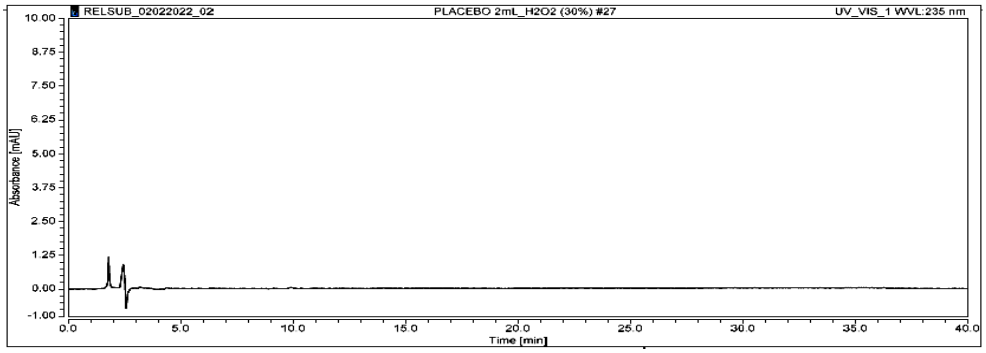

Fig. 16: Representative chromatogram of the placebo solution for the oxidation degradation test.

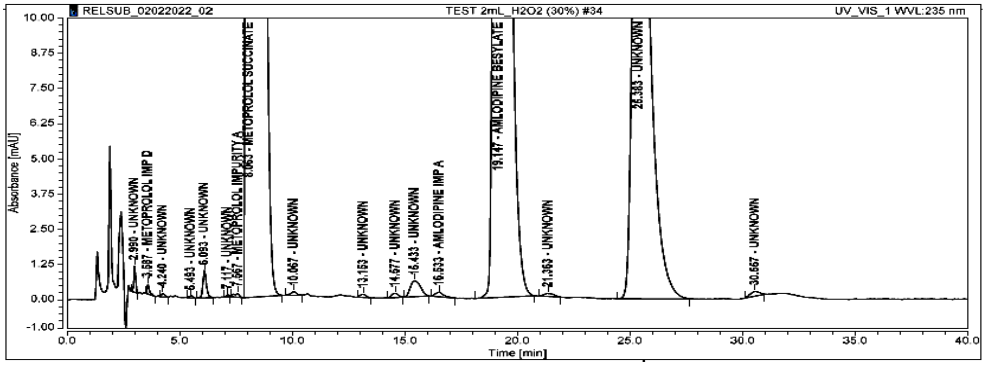

Fig. 17: Representative chromatogram of the test solution for the oxidation degradation test.

Thermal Degradation

By comparing the chromatograms of the blank, reference, test, placebo, and individual impurity solutions, no interference of any peak at the retention time of amlodipine, metoprolol, and impurities was observed. The chromatographic peaks were well separated, and there was no co-elution of impurities with the main peak and other peaks. Refer Fig. 18 to 20 for the representative chromatograms of the blank, placebo, and test solutions.

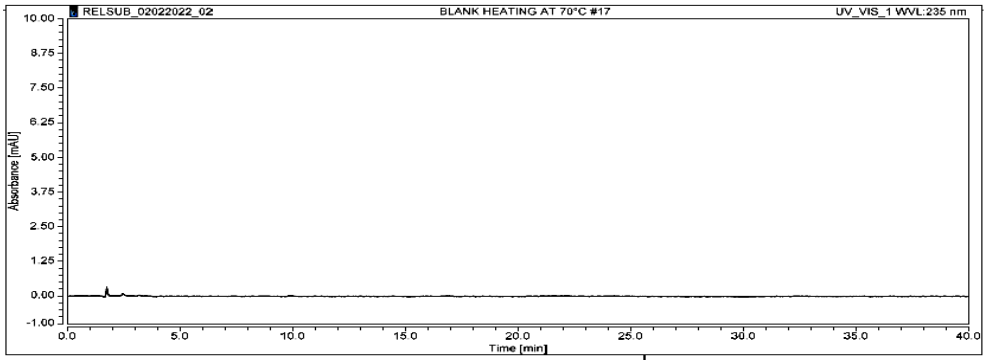

Fig. 18: Representative chromatogram of the blank solution for the thermal degradation test.

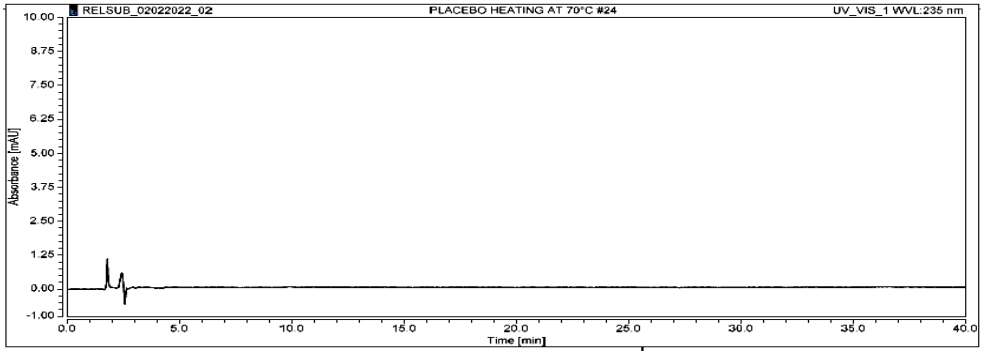

Fig. 19: Representative chromatogram of the placebo solution for the thermal degradation test.

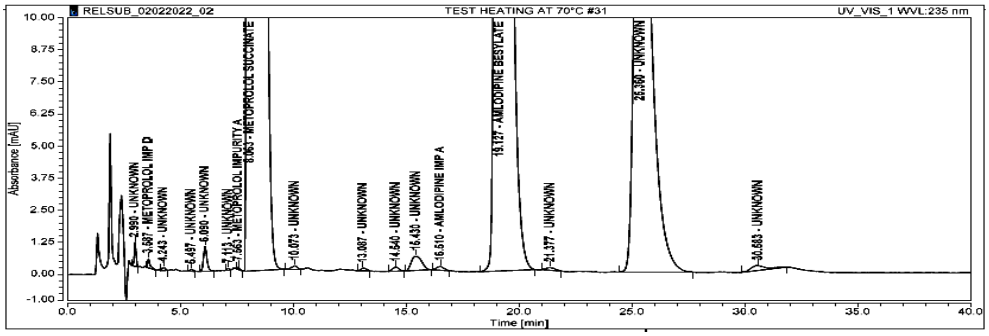

Fig. 20: Representative chromatogram the test solution for the thermal degradation test.

Photolytic degradation

By comparing the chromatograms of the blank, reference, test, placebo, and individual impurity solutions, no interference of any peak at the retention time of amlodipine, metoprolol, and impurities was observed. The chromatographic peaks were well separated, and there was no co-elution of impurities with the main peak and other peaks. Refer Fig. 21 to 23 for the representative chromatograms of the blank, placebo, and test solutions.

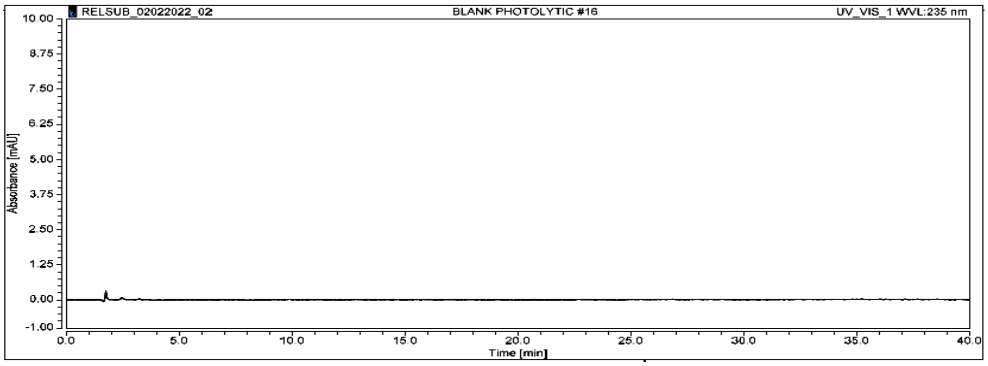

Fig. 21: Representative chromatogram of the blank solution for the photolytic degradation test.

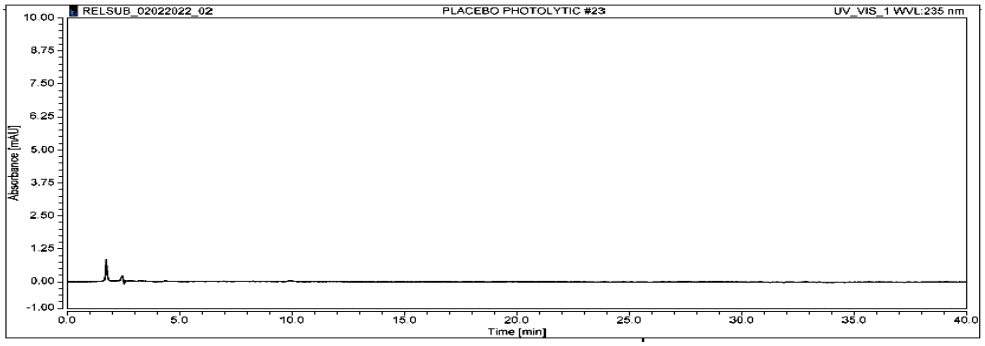

Fig. 22: Representative chromatogram of the placebo solution for the photolytic degradation test.

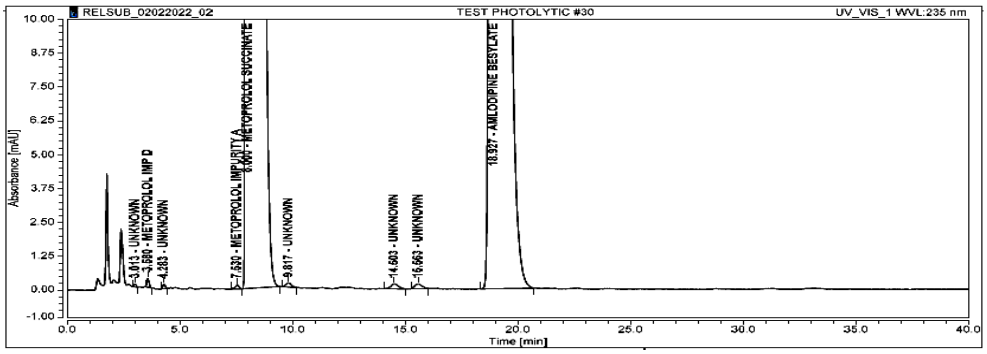

Fig. 23. Representative chromatogram of the test solution for the photolytic degradation test.

LIMITS FOR DEGRADATION

Degradation of drug substances between 5% and 20% has been accepted as reasonable for the validation of chromatographic assays.[6, 18]

The study can be terminated if no degradation is observed after the drug substance or drug product has been exposed to stress conditions than those conditions mentioned in an

accelerated stability protocol.[19] Over-stressing a sample may lead to the formation of a secondary degradation product, which may not be seen in formal shelf-life stability studies, and under-stressing may not generate sufficient degradation products.[20] A maximum of 14 d for stress testing in solution (a maximum of 24 h for oxidative tests) is recommended to provide stressed samples for method development.[21]

CONCLUSIONS

The following conclusions are drawn

1. Any interference from the peaks eluting under all the stress conditions is checked with respect to the retention time of the amlodipine and metoprolol, and no interference is observed.
2. The peak purity of amlodipine, metoprolol, and impurity A under all-stressed conditions are spectrally pure.
3. Samples undergoing acid treatment show an increase in the specified and unspecified impurities in the finished product.
4. The mass balance is achieved under all condition, which is not less than 95.0%.
5. Stability Indicating: It can be concluded from the forced degradation data presented in this study that the method for assay and related substances, that is, - specified and unspecified impurities of amlodipine and metoprolol, is specific and stability indicating. Thus, the method is selective for the estimation of amlodipine, metoprolol, and specified and unspecified impurities.

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