WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

An Investigation of Drugs Used for the Treatment of Heart Disease

Ms. Arunima Aravind V.P [a] , Ayshath Safeera.C.K [b], Mehrunnisa.P.A [b] , Fazil Jamal Mohammed[b]

Department of Pharmaceutical Analysis, Malik Deenar College of Pharmacy, Seethangoli, Bela (P.O),Kasaragod, Kerala-671321

ABSTRACT

Cardiovascular diseases remain a leading cause of morbidity and mortality globally, with myocardial infarction, heart failure, and arrhythmias being among the most critical conditions. This article explores the pharmacological management of these disorders through two key drugs: clopidogrel, and amiodarone. Clopidogrel, an antiplatelet agent, is used in myocardial infarction to prevent thrombotic events by inhibiting P2Y12 ADP receptors. Its analysis involves High-Performance Liquid Chromatography (HPLC) and LC-MS/MS, with emphasis on stability under various stress conditions.Amiodarone,apotent antiarrhythmic, acts by blocking potassium channels, thus prolonging cardiac repolarization. Analytical methods such as titrimetry, TLC, HPTLC, and gas chromatography are used to quantify amiodarone and assess its stability. The paper further discusses the structural activity relationships (SAR) and degradation profiles of these drugs, providing a comprehensive overview of their therapeutic relevance and analytical evaluation.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death globally, posing a significant burden on healthcare systems and affecting millions of individuals annually. Among these, myocardial infarction, heart failure, and cardiac arrhythmias represent some of the most severe and life-threatening conditions. The pharmacological management of these disorders is crucial for improving patient outcomes and preventing complications. This article focuses on two essential drugs widely used in the treatment of cardiovascular conditions: clopidogrel, and amiodarone. Clopidogrel is a key antiplatelet agent utilized in the management of myocardial infarction; and amiodarone serves as a potent antiarrhythmic medication effective in both atrial and ventricular arrhythmias. In addition to their therapeutic applications, this paper also explores the synthesis, analytical methods, structural activity relationships, and stability profiles of these drugs, providing a comprehensive overview of their pharmacological and pharmaceutical significance

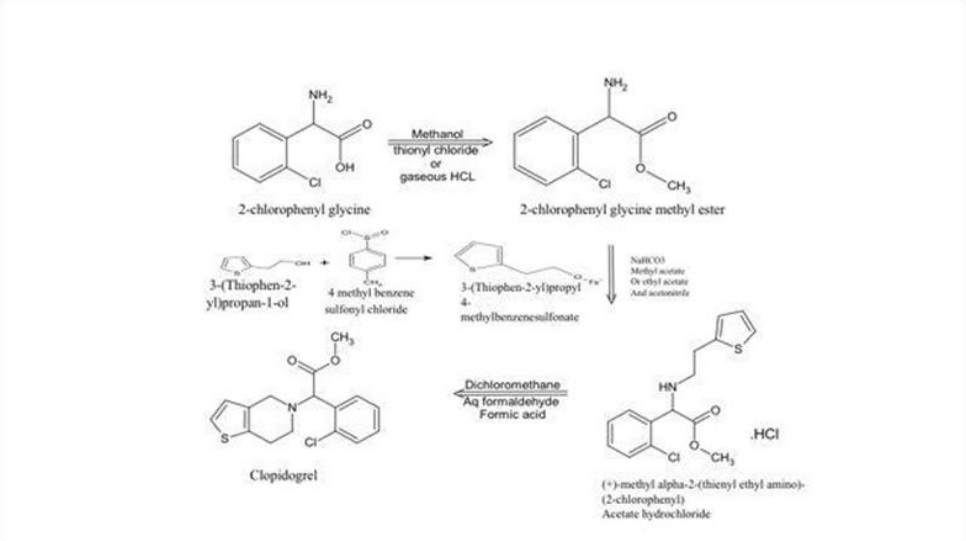
DRUGUSED IN MYOCARDIAL INFRACTION

Heart attacks, sometimes referred to as myocardial infarctions, are dangerous medical conditions in which a section of the heart muscle is injured or dies as a result of inadequate oxygen and blood supply. This occurs when a blood clot or plaque accumulation blocks one of the coronary arteries that provide blood to the heart.[1]

CLOPIDOGREL

People who are at a high risk of cardiovascular events, such as heart attacks and strokes, can use the medicine clopidogrel to prevent blood clots. Clopidogrel belongs to a group of drugs known as antiplatelet drugs. It functions by keeping platelets, a kind of blood cell, from clumping together and creating clots that could result in a heart attack.[2] A two-step reaction with an active thiol-containing metabolite activates clopidogrel[3]. The P2Y12 ADP receptors on platelets are irreversibly bound by this active form, which is a platelet inhibitor.9. This interaction stops platelet aggregation, the activation of the glycoprotein GPIIb/IIIa complex, and ADP binding to P2Y12 receptors.[4]

SYNTHESIS



High-Performance Liquid Chromatography (HPLC))

Clopidogrel analysis using High-Performance Liquid Chromatography (HPLC) usually starts with the production of standard and sample solutions. Weigh the clopidogrel standard precisely, dissolve it in methanol, and then dilute it with the mobile phase to the desired concentration. Likewise, make the sample by using methanol to extract a precisely weighed amount of the powdered tablet that is equal to a predetermined amount of clopidogrel, then sonicating it for 10 to 15 minutes and filtering it through a 0.45 µm membrane filter. A reversed-phase C18 column (250 mm × 4.6 mm, 5 µm) and a UV detector positioned at about 220–254 nm are included in the chromatographic system. At room temperature, the mobile phase is typically a 70:30 v/v combination of acetonitrile and water or a buffer (such as phosphate buffer pH 3.0) injected at a flow rate of 1.0 mL/min. The standard and sample solutions are injected separately (usually 10–20 µL) after the baseline has stabilized, and the chromatograms are recorded for roughly 10–15 minutes per run. The peak area of clopidogrel is measured after it elutes at its typical retention time, which is around 6 to 8 minutes.[5]

Liquid Chromatography-Mass Spectrometry (LC-MS / LC-MS/MS)

Detection of Clopidogrel and its metabolites, particularly the active thiol metabolite, is extremely sensitive and specific. To guarantee compatibility with both the LC and MS systems, the preparation of a Liquid Chromatography-Mass Spectrometry (LC-MS) analysis usually starts with the careful selection of suitable solvents, standards, and sample solutions. To improve ionization, the target analyte is first precisely weighed and dissolved in an appropriate solvent, such as methanol, acetonitrile, or water, frequently with a volatile addition like ammonium acetate or formic acid. Detection of Clopidogrel and its metabolites, particularly the active thiol metabolite, is extremely sensitive and specific. To guarantee compatibility with both the LC and MS systems, the preparation of a Liquid Chromatography-Mass Spectrometry (LC-MS) analysis usually starts with the careful selection of suitable solvents, standards, and sample solutions. In order to improve ionization, the target analyte is first precisely weighed and dissolved in an appropriate solvent, such as methanol, acetonitrile, or water, frequently with a volatile addition like ammonium acetate or formic acid.[6]

STABILITY TEST

To ascertain how the quality of the drug ingredient or product changes over time under the effect of environmental conditions like temperature, humidity, and light, stability studies of clopidogrel are crucial. These investigations guarantee shelf-life, safety, and effectiveness.[7]

Acid hydrolysis

Clopidogrel is frequently acid hydrolyzed by subjecting it to a strong acid, usually diluted hydrochloric acid (0.1–1 N HCl), while it is being heated or refluxed under controlled conditions. This process breaks down the molecular structure of clopidogrel into its corresponding acid, alcohol, and other degradation products by hydrolytically cleaving its ester and/or other acid-labile functional groups. The clopidogrel-acid combination is typically heated to 60–80 °C for a predetermined amount of time, usually one to four hours, in order to carry out the reaction. To identify and measure the degradation products, the mixture is neutralized, extracted, and analyzed when the reaction is finished, frequently using methods like HPLC, LC–MS, or TLC.[7]

Basic hydrolysis

Clopidogrel undergoes basic hydrolysis when it is heated or refluxed with a strong base, usually sodium hydroxide or potassium hydroxide (e.g., 0.1–1 N NaOH). The ester group in clopidogrel's molecular structure is hydrolytically cleaved by the alkaline environment, forming an alcohol and carboxylic acid moiety in the process. Clopidogrel is usually combined with the alkaline solution after being dissolved in an appropriate solvent, such as methanol or acetonitrile. Before additional examination, the reaction mixture is often heated to 60–80°C for a few hours (for example, one to four hours), cooled, and neutralized with a diluted acid.[7]

Thermal Degradation

By subjecting the drug or its solid dosage form to high temperatures, usually between 60°C and 105°C, for a predetermined amount of time—typically a few hours to several days—the thermal degradation of clopidogrel is examined. Clopidogrel may slowly break down under these circumstances, changing its chemical structure by breaking down its ester moiety and forming degradation products such carboxylic acid or other similar chemicals. To determine the degree of deterioration and find any products of thermal degradation, samples are taken out at different intervals and examined using methods like HPLC or LC-MS.[7]

Photolytic Degradation

In accordance with ICH guidelines (e.g. ICH Q1B), photolytic degradation of clopidogrel is investigated by subjecting the medication to direct light sources, such as UV and visible light. Usually, clopidogrel powder or its solution is applied thinly or stored in a clear container, then exposed to controlled light stress for a predetermined amount of time (e.g., 1.2 million lux hours and 200 watt-hours/m² UV). Clopidogrel's photosensitive functional groups may break down when exposed to light, resulting in structural alterations and the production of photodegradation products. Following exposure, the samples are examined using LC-MS, HPLC, or other appropriate analytical techniques to find any new peaks or assay modifications.[7]

ARRHYTHMIA

Abnormal heartbeats are known as arrhythmias. Any deviation from the typical electrical impulse sequence is referred to as a "arrhythmia." An irregular heartbeat is caused by electrical impulses that are either too rapid, too slow, or chaotic during an arrhythmia.

Adults with a rapid heart rate (more than 100 beats per minute) are said to have tachycardia. Bradycardia is the term for a sluggish heart rate (less than 60 beats per minute).[11]

TYPES OF ARRHYTHMIAS

1. **Bradycardia**
2. **Tachycardia**
3. **A premature or extra heartbeat**

Supraventricular arrhythmias This type of arrhythmia starts in the atria or the gateway to the lower chambers

i. **Atrial fibrillation**

ii. **Atrial flutter**

iii. **Paroxysmal supraventricular tachycardia (PSVT)**

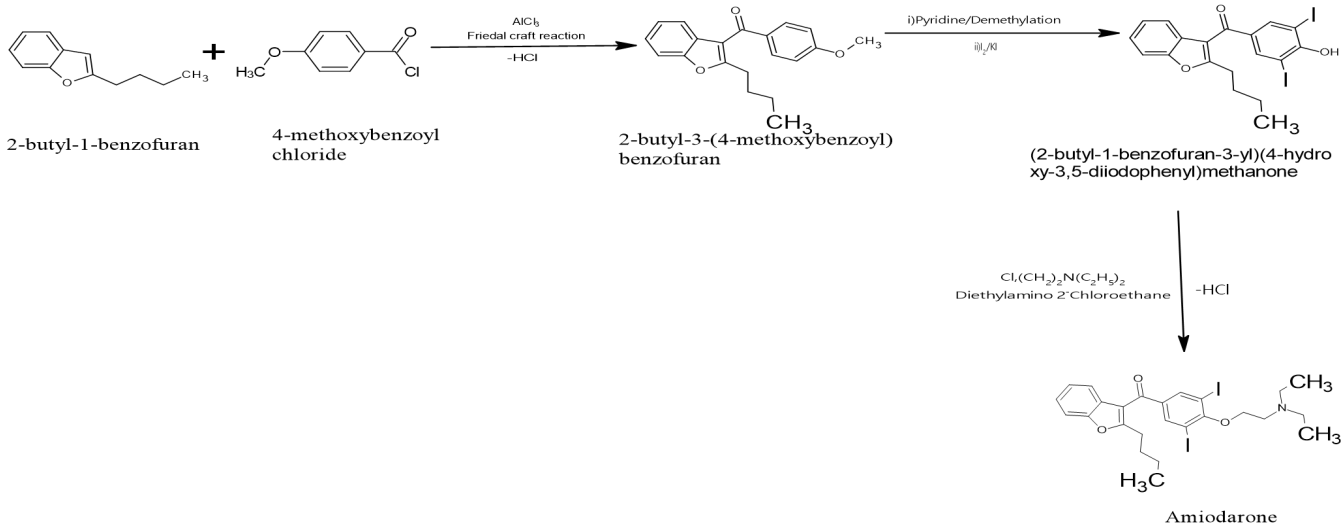
Ventricular arrhythmias Ventricular arrhythmias start in your heart’s lower chambers, called the ventricles.

- **Ventricular tachycardia**
- **Ventricular fibrillation**

AMIODARONE

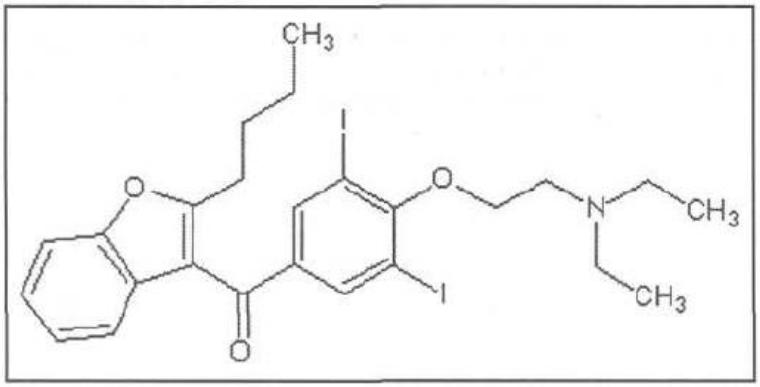
Amiodarone is an antiarrhythmic drug with complicated antiarrhythmic properties, include blocking a number of ion channels, primarily potassium and, to a lesser extent, sodium channels. For many years, this medication has been used to treat patients with ventricular and atrial arrhythmias.[9]

SYNTHESIS OF AMIODARONE



After 2-butylbenzofurane is acylated with 4-methoxybenzoyl chloride to produce 2-butyl-3-(4-methoxybenzoyl) benzofuran, the product is demethylated by pyridine hydrochloride and iodised with potassium iodide to produce 2-butyl-3-benzofuranyl-4-(2-hydroxy-3,5-diiodophenyl) ketone. This compound then reacts with 2-diethylaminoethylchoride to yield amiodarone.

SAR OF AMIODARONE



1. Benzofuran Ring System: Activity requires butyl benzofuran. Methyl sulphonamide substitution could boost the activity. For instance: -Dronedarone
2. Iodine Substituents: Activity requires diiodo substitution on the benzene ring. Iodine removal has negative consequences.

3. Alkyl Side Chain: A longer chain could result in a longer half-life. It is possible to substitute a bigger alkyl group for one of the ethyl replacements.

4. Tertiary Amine Group: When a tertiary amino group is transformed into a secondary amine, its activity remains unchanged, but its toxicity increases.[14]

MECHANISM OF ACTION

Amiodarone is a potent potassium channel blocker. It prevents potassium currents from causing the heart muscle to repolarise during the cardiac action potential's third phase. Amiodarone hence lengthens the action potential's duration and the cardiac cells' (myocytes') effective refractory period. Consequently, aberrant heart rhythms are prevented and treated by lowering the excitability of cardiac muscle cells. [10]

Methods of Analysis of Amiodarone

Drug products including antiarrhythmic medicines are quantitatively estimated using a variety of simultaneous estimating techniques, including spectrophotometric, High Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC).

- **TITRIMETRIC ANAYLSIS**

1) Aqueous

500 mg of a precisely weighed sample of amiodarone hydrochloride should be dissolved in 5 ml of methanol and then diluted with distilled water to make 50 ml. Five millilitres of the solution should be pipetted into a 100-millilitre titration vessel with a stopper. Twenty millilitres of distilled water, five millilitres of 2 M sulphuric acid, one millilitre of indicator solution (0.01% dimethyl yellow in ethanol), and twenty millilitres of chloroform should then be added. After adding 25 millilitres of a 0.12% sodium dioctyl sulphosuccinate (D.O.S.S.) solution and shaking the mixture violently with a burette, a yellow layer of chloroform was visible. Following each addition, titration is carried out using amounts of 0.1 to 0.2 ml of D.O.S.S. and vigorous shaking. The change in the colour of the chloroform layer from yellow to red-orange indicates the titration's end-point. Amiodarone hydrochloride is equal to 18.41 mg per millilitre of 0.12% (0.027 mol) D.O.S.S.[15]

2.Non-aqueous Titration

Accurately weigh out 60 mg of amiodarone hydrochloride and dissolve it in 50 ml of glacial acetic acid and 10 ml of a 3% mercuric acetate in acetic acid solution. To modify the solution's colour from blue to lilac, add five drops of a 0.5% solution of Oracet Blue B in acetic acid and titrate with standardised 0.1 N perchloric acid in acetic acid. Proceed with a blank determination and make any required adjustments.Amiodarone hydrochloride has a molecular weight of 68.18 mg per millilitre of 0.1 N perchloric acid. The titration with perchloric acid described above can be used in the same manner to assay desethylamiodarone hydrochloride. 65.38 mg of desethylamiodarone hydrochloride are equal to one millilitre of 0.1 N perchloric acid.[15]

II Chromatographic Analysis

1. Thin-Layer Chromatography

The following system has been used to report a thin-layer chromatographic (TLC) assay of amiodarone and some of its analogues in raw material. Absorbent Microscopic Silica Gel GF, 250

Activation: 30 minutes of plate drying at 110°C

Eluting solvent: 90:7:3 v/v of chloroform, methanol, and formic acid

Chloroform concentration: 50 mg/ml

One hour of equilibration in a closed tank coated with paper

Iodine absorption and ultraviolet light at 254 nm are used for detection.

Spotting: 250-500 mg of amiodarone hcl ,1.25 µg of amiodarone analogues

The plate is taken out of the chromatographic chamber and dried in an air current until there is no more solvent odour visible after the solvent has been allowed to rise to the 15 cm line. After being analysed at 254 nm by UV light, the plate is left in an iodine crystal chamber for approximately half an hour. After being exposed to iodine, all items that had darkening blue spots under UV light became yellow-brown blotches. Amiodarone's Rf is 0.28, while those of its counterparts, L3937, L6355, L3372, L6424, and L3373, are, respectively, 0.17, 0.23, 0.53, 0.62, and 0.73.[15]

2. High-Performance Thin-Layer Chromatography

Drug chromatographic separation was carried out on aluminium plates that had been previously coated with silica gel 60 GF254 (10 cm × 10 cm with a layer thickness of 250 µm). Using a Camag 100 µl sample syringe and a Linomat 5 applicator, the sample was placed to the plate in a band that was 4 mm wide. The mobile phase was made up of 9:1 v/v ethyl acetate and methanol. The TLC plate was developed linearly rising under 15-minute saturation conditions in a 10 cm × 10 cm Camag twin trough glass chamber using 10 ml of mobile phase per pass. The distance of migration was 80 mm.

Using a Camag TLC scanner 3 with a slit measuring 3.00 x 0.45 mm and a deuterium lamp as a radiation source, densitometric scanning was carried out in the 200–400 nm range using Win CATS software.[16]

- **Gas Chromatography**

This technique uses a glass column filled with 2.5% SE 30 on 80–100 mesh Chromosorb G and a flame ionisation detector to identify amiodarone. Nitrogen was utilised as the carrier gas at a flow rate of 45 millilitres per minute, and the column temperature was fixed at 300 degrees Celsius. Under these circumstances, drug retention indices of 3335, 2590, and 2780 were found for amiodarone and two significant on-column breakdown products, respectively. Amiodarone, desethylamiodarone, and six amiodarone analogues were subjected to their own gas chromatographic analyses on two glass columns filled with 3% OV17 on 80-100 mesh Gas Chrom Q and 3% OV17 on 80-100 mesh Gas Chrom Q. A flame ionisation detector (FID) and a 63Ni electron capture detector were used for both columns. An injector temperature of 25°C, a detector temperature of 280°C for the FID and s3Ni detectors, and a nitrogen carrier gas flow of 30 ml/min were used for both columns. Amiodarone and its analogues were screened gas chromatographically using column temperatures of 100°C, 120°C, 140°C, 180°C, 210°C, 230°C, and 250°C. The chromatograms of all compounds at all chosen column temperatures showed no peaks within an hour after the injection of 1µl of methanolic standard solutions containing 1 mg/ml of amiodarone and its analogues. This absence of reaction could be explained by the fact that, according to the melting point determination of these compounds, amiodarone and its analogues suffer prolonged thermal degradation with evolution of iodine at temperatures between 150°C and 200°C.[15]

STABILITY STUDIES OF AMIODARONE

1) Alkaline hydrolysis

To 1 ml stock solution of Amiodarone hydrochloride (1000 µg/ml), 1 ml of 1 N NaOH was added. The aforementioned solution was stored at room temperature for three hours. After applying 4 µl of the resulting solution to the TLC plate, a densitogram was created. Without a degradation peak, an average of 68.97% of amiodarone hydrochloride was recovered.[11]

2) Acid hydrolysis

One millilitre of 0.5 N HCl was added to a stock solution of amiodarone hydrochloride (1000 µg/ml). The aforementioned solution was stored at room temperature for three hours. After applying 4 µl of the resulting solution to the TLC plate, a densitogram was created. Amiodarone hydrochloride was recovered at an average rate of 88.63% without a degradant peak.[11]

3) Oxidative degradation

One millilitre of 30% H2O2 was added to a millilitre of amiodarone hydrochloride stock solution (1000 µg/ml). The aforementioned solution was stored at room temperature for three hours. After applying 4 µl of the resulting solution to the TLC plate, a densitogram was created. Amiodarone hydrochloride was recovered at an average rate of 88.03% without a degradant peak.[11]

4) Photodegradation studies

The medication was exposed to UV light with NLT 200-watt hr/m2 irradiation to examine its photodegradation. A precisely weighed 10 mg dose of the medication was added to a 10 ml volumetric flask after exposure, and the volume was adjusted with methanol to achieve 1000 µg/ml. To achieve a 500 µg/ml concentration, 5 ml of the resulting solution was subsequently diluted with methanol. After applying 4 µl of the resulting solution to the TLC plate, a densitogram was created. With no degradant peak, an average of 95.15% of amiodarone hydrochloride was recovered.[12]

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all those who contributed to the successful completion of this article. My heartfelt thanks go to [Your Institution/Department Name] for providing the necessary resources and a supportive academic environment.

I extend my special thanks to my mentor, [Mentor's Name], for their valuable guidance, insightful suggestions, and continuous encouragement throughout the development of this work. Their expertise greatly enriched the content and analytical approach of this study.

I am also grateful to my colleagues and peers for their constructive feedback and motivation, which helped improve the quality of this article. Lastly, I wish to acknowledge the authors and researchers whose work has been cited, forming the foundation of this comprehensive analysis on the pharmacological and analytical evaluation of clopidogrel, digoxin, and amiodarone.

REFERENCE

1 Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001;345(7):494–502.[1]

2 Savi P, Pereillo JM, Uzabiaga MF, et al. Identification and biological activity of the active metabolite of clopidogrel. *Thromb Haemost*. 2000;84(5):891–6.[2]

3 Shrivastava A, Gupta VB. Methods for the determination of clopidogrel in pharmaceutical and biological samples: a review. *J Pharm Biomed Anal*. 2012;55(4):775–87.[3]

4 ICH Q1A(R2). Stability Testing of New Drug Substances and Products. International Conference on Harmonisation; 2003.[4]

5 Martindale: The Complete Drug Reference. 38th ed. London: Pharmaceutical Press; 2014.[5]

6 Goldberger AL. Clinical Electrocardiography: A Simplified Approach. 8th ed. Elsevier Health Sciences; 2012.[6]

7 Sweetman SC, editor. Martindale: The Complete Drug Reference. 36th ed. London: Pharmaceutical Press; 2009.[7]

8 Textbook of medicinal chemistry by Ilango,volume 1,page no 256 8

12 Chugh SS, Reinier K, Singh T, Uy-Evanado A, Socoteanu C, et al. Determinants of prolonged QT interval and their contribution to sudden death risk in coronary artery disease: the Oregon Sudden Unexpected Death Study. *Circulation*. 2009;119(5):663–70.[12]

9 Singh BN. Amiodarone: Historical development and pharmacologic profile. *Am Heart J*. 1983;106(4):788–97.[9]

10 Tripathi KD. Essentials of Medical Pharmacology. 8th ed. Jaypee Brothers Medical Publishers; 2018.[10]

11 Basak A, Rawat PS, Patel SS. Stability indicating chromatographic methods for estimation of amiodarone hydrochloride. *J Pharm Res*. 2011;4(12):4407–9.[15]

12 ICH Q1B. Photostability Testing of New Drug Substances and Products. International Conference on Harmonisation; 1996.[12]

13 Sharma MC, Sharma S. UV Spectrophotometric and Stability Studies of Amiodarone Hydrochloride. *Int J Pharm Sci Rev Res*. 2010;2(2):59–62.[13]

14 Siddiqui N, Ahsan W, Mishra A. A review of structure activity relationship of amiodarone and its derivatives. Pharmacology & Pharmacy. 2016;7(3):108–12. doi:10.4236/pp.2016.[14]

15 Cervelli JA, Kerkay J, Pearson KH. Internal titrimetric, TLC and GC methods for amiodarone hydrochloride analysis. Unpublished; as described in pharmacopoeial or lab-specific methodology.

16 Silva RL, Junqueira RG, Souza SVC, et al. Stability-indicating HPTLC and HPLC methods for determination of amiodarone hydrochloride in tablets. *Braz J Pharm Sci*. 2020;56.