IJBPAS, August, 2026, 15(8): 3578-3605

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ADVANCING GREEN PHARMACEUTICAL ANALYSIS: A CRITICAL ASSESSMENT OF AMIODARONE ESTIMATION METHODS WITH INTEGRATION OF GAPI AND AGREE METRICS

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Received 26th June 2025; Revised 10th Aug. 2025; Accepted 16th Oct. 2025; Available online 1st Aug. 2026

https://doi.org/10.31032/IJBPAS/2026/15.8.10334

ABSTRACT

The increasing use of sophisticated analytical techniques and organic solvents has sparked growing environmental concerns due to their ecological footprint. Amiodarone, a potent antiarrhythmic agent widely prescribed for managing various cardiac arrhythmias, has been extensively studied through diverse analytical methodologies to ensure accurate quantification in bulk and pharmaceutical formulations. This review systematically compiles and evaluates articles focused on the analytical determination of Amiodarone using techniques such as UV spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS), Capillary electrophoresis (CE) and liquid chromatography–mass spectrometry (LC-MS). Special attention is given to the nature of solvents used and the environmental impact of these procedures. Greenness assessment tools, including Green Analytical Procedure Index (GAPI), and AGREE metrics, are employed to assess the ecological compatibility of each technique. This study aims to guide the development and selection of eco-friendly analytical techniques that maintain reliability in Amiodarone analysis while minimizing environmental harm.

Keywords: Amiodarone, Antiarrhythmic drugs, HPLC, UPLC-MS, LC-MS/MS, UV spectrophotometry, Greenness assessment, GAPI, AGREE

1. INTRODUCTION

In pharmaceutical sciences and drug development, the precise determination of drug concentrations is essential to guarantee the therapeutic effectiveness, patient safety, and consistency in quality of medicinal products. A broad spectrum of analytical methods has been established to accommodate the varying requirements of different drugs and regulatory frameworks. These methods are crucial not only for the quantification of active pharmaceutical ingredients (APIs) but also for identifying impurities, degradation compounds, and excipients. The formulation and validation of dependable analytical procedures are necessary at every stage of drug development, ranging from initial formulation design to large-scale manufacturing and final quality assurance. Additionally, such methods support critical evaluations including pharmacokinetic profiling, stability testing, and bioavailability assessments, thereby strengthening the overall scientific and regulatory foundation of drug products. Green Analytical Chemistry (GAC) promotes sustainable practices through miniaturization, solventless or solvent-minimized techniques, and the use of less hazardous reagents to reduce environmental impact **[32]**.

1.1. Amiodarone and Cardiac Arrhythmias: A Therapeutic and Analytical Overview

Amiodarone plays a pivotal role in treating a wide range of cardiac arrhythmias, particularly when other antiarrhythmic drugs prove ineffective or poorly tolerated. It is uniquely effective against a broad spectrum of arrhythmias, including ventricular tachyarrhythmias, atrial fibrillation, and supraventricular tachycardias. Its mechanism is complex, involving multiple electrophysiological pathways most notably, the prolongation of repolarization across myocardial tissue, which helps suppress ectopic activity and reentrant circuits.

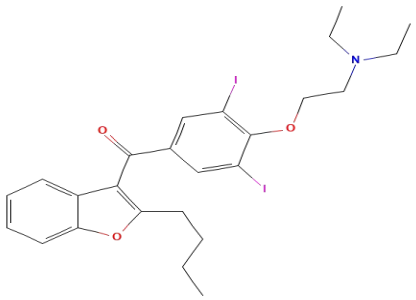


Figure 1: Structure of Amiodarone

The clinical utility of amiodarone stems not only from its broad antiarrhythmic coverage but also from its efficacy in otherwise drug-resistant cases. It is especially valuable in high-risk patients where arrhythmia control is critical, such as those with ischemic heart disease or structural cardiac abnormalities. However, its use is tempered by its slow onset of action, prolonged half-life, and potential for serious adverse effects involving the lungs, thyroid, liver, and eyes, necessitating regular monitoring during long-term therapy.

From an analytical standpoint, the lipophilic nature of amiodarone and its extensive tissue binding pose challenges in pharmacokinetic studies and therapeutic drug monitoring. These complexities have driven the development of various sensitive and specific analytical methods to ensure accurate measurement of the drug in biological matrices and pharmaceutical formulations, which is essential for optimizing clinical outcomes and minimizing toxicity risks **[1]**. Amiodarone Hydrochloride, a Class III antiarrhythmic agent, prolongs cardiac repolarization by affecting phase 3 of the action potential and is characterized by poor water solubility, a pKa of 6.56, and a melting point of 158–165 °C **[33]**.

1.1.1. Mechanism Of Action:

Amiodarone exhibits a multifaceted mechanism of action, acting on a range of ion channels and receptors that regulate cardiac electrophysiology. It is primarily classified as a Class III antiarrhythmic agent, due to its capacity to prolong the Period of myocardial depolarization and repolarization and refractory period in myocardial tissue. However, it also displays properties associated with Class I, II, and IV agents.

In the acute phase, Amiodarone suppresses inward sodium (INa) and calcium (ICa) currents, with inhibition varying based on use and membrane voltage. This leads to suppressed excitability and reduced conduction velocity, particularly in rapidly firing cardiac tissues or those with depolarized membrane potentials. Inhibition of outward potassium currents also occurs, including various types such as IKr (rapid delayed rectifier), IKs (slow delayed rectifier), IK,ACh (acetylcholine-sensitive), and IK,Na (sodium-activated potassium current). At therapeutic levels, this multi-channel blockade contributes to antiarrhythmic effects without causing excessive proarrhythmia.

With prolonged administration, amiodarone consistently induces moderate prolongation of the APD across different cardiac tissues which shows weak rate-dependence. This is largely attributed to the reduction in charge

flow density of potassium channels (especially IKr and Ito), and partially from downregulation of Kv1.5 gene expression, indicating potential modulation at the genomic level. Additionally, the drug and its active metabolite, desethylamiodarone, accumulates in cardiac tissues and continues to exert prolonged inhibitory effects.

Amiodarone also exerts noncompetitive beta-adrenergic blockade and calcium channel inhibition, and interferes with thyroid hormone metabolism at the cardiac level. It has been observed to antagonize triiodothyronine (T3) effects, leading to physiological changes in the heart that resemble hypothyroidism, such as bradycardia and prolonged repolarization **[2]**.

1.1.2. Chemical and Pharmacological Profile:

The chemical name of Amiodarone is 2-butyl-3-(3,5-diiodo-4-hydroxybenzoyl)-1-benzofuran

-5-yl]-[4-(diethylamino)butyl]methanone.

Amiodarone is a benzofuran derivative, originally synthesized in 1962 by Labaz Laboratories in Belgium as part of a broader search for coronary vasodilators. Its structure includes a benzofuran ring and two iodine atoms covalently bonded to an aromatic system, making it structurally related to natural compounds like khellin and its derivatives.

Table 1: Characterization of physicochemical properties of Amiodarone

Parameter	Details
CAS Number	1951-25-3
Molecular Formula	$C_{25}H_{29}I_2NO_3$
Molecular Weight	645.32 g/mol
Appearance	White to off-white crystalline powder
Melting Point	155–160 °C
Solubility	Soluble in ethanol, chloroform, and methanol; slightly soluble in water

The iodinated nature of amiodarone significantly contributes to its high lipid solubility, large volume of distribution, and interaction with thyroid hormone metabolism, which later became evident in clinical observations. Initially, its precursor compound, benziodarone, was discarded due to hepatotoxicity, while amiodarone emerged with a stronger pharmacological profile and better tolerability **[3]**.

Table 2: Characterization of Pharmacological properties of Amiodarone

Property	Effect
Electrophysiology	Prolongs APD and ERP, minimal effect on depolarization/conduction
Adrenergic Receptors	Noncompetitive α and β blockade
Coronary/Peripheral Vasodilation	Reduces vascular resistance; improves coronary blood flow
Inotropic Effect	Mild to negligible negative inotropy at clinical doses
Thyroid Interaction	Antagonizes T3 effects; alters thyroid hormone metabolism
Onset/Offset	Delayed onset, long half-life, sustained tissue binding

Amiodarone displays a unique combination of electrophysiological, antiadrenergic, and vasodilatory properties, making it distinct among antiarrhythmic agents. Its key pharmacological characteristics include:

- Electrophysiological Effects: Amiodarone prolongs action potential duration and refractory period, especially with chronic use, without significantly affecting depolarization or conduction velocity. Unlike Class I agents, it does not depress contractility, and conduction issues are rare unless pre-existing **[3]**.
- Adrenergic Blockade: It noncompetitively blocks alpha- and beta-receptors, suppressing adrenergic tachycardia and preventing reflex responses to vasodilation **[3]**.
- Vasodilation: Acts as a potent coronary and peripheral vasodilator, lowering systemic resistance and enhancing perfusion without reflex sympathetic activation **[3]**.
- Inotropic Profile: Shows minimal negative inotropic effect at therapeutic doses; reduced afterload from vasodilation may offset any mild contractility decline **[3]**.
- Thyroid Interaction: Due to its iodine-rich structure, it alters thyroid hormone metabolism and antagonizes T3 in cardiac tissue, contributing to its prolonged cardiac effects **[3]**.
- Multiple Dosing/Drug-Drug Interactions:
    - A preclinical study in normal rabbits demonstrated that multiple-dose administration of Amiodarone significantly enhanced the onset, peak, and duration of hypoglycemia induced by Pioglitazone and Nateglinide. This suggests a pharmacokinetic interaction that warrants caution in diabetic patients with coexisting arrhythmias **[34]**.
    - Drug interactions, particularly involving agents like Amiodarone and Levofloxacin, may significantly impact therapeutic outcomes due to altered metabolism, protein binding, or renal elimination. Analytical techniques such as HPLC and LC-MS have been widely validated for quantifying Amiodarone, its

metabolites, and related impurities, making them essential tools in drug interaction and pharmacokinetic studies **[35]**.

1.1.3. Pharmacokinetic profile:

Absorption: Oral bioavailability ranges between 35% and 65%, with significant and variable gastrointestinal absorption **[4]**.

Distribution: Amiodarone's lipophilicity leads to substantial accumulation in various body tissues., leading to an exceptionally large interindividual variation in tissue uptake. It has a large volume of distribution, reflecting its deep sequestration in tissues **[4]**.

Elimination Half-Life: The plasma half-life after IV administration is relatively short (reflecting redistribution), but with prolonged oral administration, the terminal elimination half-life may extend to as long as 60 days, and varies between individuals **[4]**.

Elimination Pathways: Amiodarone is extensively metabolized to desethyl-amiodarone, an active metabolite. Plasma concentrations of this metabolite may surpass the parent drug with long-term usage **[4]**. Plasma concentrations exceeding 2.5 mg/L are correlated with greater toxicity potential **[4]**.

Loading and Maintenance Dosing: Due to its slow accumulation, it can take several weeks to months to reach steady-state tissue levels, unless a high initial loading dose (800-1600 mg/day) is used **[4]**.

1.1.4. Fixed-Dose Combinations (FDCs): Clinical Relevance and Analytical Implications

Unlike many therapeutic agents used in chronic conditions, Amiodarone is predominantly prescribed as monotherapy due to its complex pharmacokinetic profile, high tissue accumulation, and narrow therapeutic index. Currently, there are no widely marketed or approved fixed-dose combinations (FDCs) containing Amiodarone. The absence of FDCs is primarily attributed to the need for individualized dosing regimens, especially during the loading and maintenance phases of therapy.

However, in clinical practice, Amiodarone is frequently co-administered with other cardiovascular drugs to manage coexisting conditions such as hypertension, heart failure, or atrial fibrillation. These include:

- Amiodarone + Beta-blockers (e.g., Metoprolol or Carvedilol): For enhanced rate control in atrial fibrillation or post-myocardial infarction settings.

- Amiodarone + Anticoagulants (e.g., Warfarin or NOACs): For stroke prevention in atrial fibrillation.
- Amiodarone + ACE inhibitors/ARBs: In cases of heart failure with arrhythmia burden.

Although these combinations are not available as single-pill FDCs, their simultaneous use poses challenges in terms of drug-drug interactions, toxicity monitoring, and analytical quantification in multi-drug regimens.

Given the widespread co-administration of Amiodarone with other cardiovascular agents, the need for precise, selective, and robust analytical methods becomes crucial. These methods must accurately quantify Amiodarone in the presence of other drugs and excipients, both in bulk formulations and in biological matrices during therapeutic drug monitoring.

This review compiles and critically analyzes the analytical techniques established for the quantification of Amiodarone, including:

- UV-Visible Spectrophotometry
- High-Performance Liquid Chromatography (HPLC)
- Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS)
- High-Performance Thin Layer Chromatography (HPTLC)
- Liquid Chromatography–Mass Spectrometry (LC-MS/MS)

Each method is evaluated based on analytical performance parameters (accuracy, sensitivity, specificity) and environmental sustainability, applying Sustainability evaluation frameworks such as the Green Analytical Procedure Index (GAPI), and AGREE metric.

As the pharmaceutical industry moves toward greener practices, incorporating Green Analytical Chemistry (GAC) principles into analytical optimization has become imperative. This review emphasizes the importance of selecting eco-friendly yet reliable techniques for Amiodarone analysis and identifies research gaps where greener innovations are still needed.

2. Greenness Evaluation:

The pharmaceutical, agrochemical, food, petrochemical, and biotechnological industries are among the major contributors to analytical waste, which poses a significant threat to environmental sustainability. In many regions, this impact is exacerbated by inadequate regulatory oversight, making the environmental burden comparable to that observed even in highly regulated countries **[5]**.

Analytical scientists across various industrial sectors have consistently aimed to design robust and reliable methods that

comply with evolving regulatory expectations. In recent years, regulatory bodies have increasingly emphasized the adoption of environmentally sustainable analytical practices. This shift seeks to Suppress or exclude the release of harmful effluents generated by the widespread use of chemical solvents, reagents, and other potentially polluting substances within analytical laboratories **[6]**.

To assess the ecological compatibility of reported quantitative approaches, this review employs well-established tools from the domain of Green Analytical Chemistry (GAC), including the Analytical Greenness Metric Approach (AGREE), and Green Analytical Procedure Index (GAPI). These software-based tools were applied to the selected methods to evaluate their ecological impact. The review critically analyzes a range of modern analytical techniques designed for the quantification of Amiodarone in both bulk drug and pharmaceutical preparations, with a particular focus on balancing analytical performance and environmental responsibility.

2.1. Green Analytical Procedure Index (GAPI)

The Green Analytical Procedure Index (GAPI) is a qualitative assessment tool developed to assess the ecological footprint of an analytical method across its entire lifecycle from the collection of samples to final outcomes. It utilizes a distinctive pictogram divided into 15 pentagonal sections, each corresponding to a specific step in the analytical workflow. Unlike binary green/non-green schemes, GAPI uses a three-level chromatic scale:

- Green for low environmental footprint,
- Yellow for moderate footprint, and
- Red for high environmental burden.

This color scheme visually conveys the overall greenness of a method, factoring in aspects such as solvent usage, sample preservation, reagent toxicity, sample preparation complexity, transport, storage conditions, and energy consumption.

To further quantify the risk associated with each solvent used in the reviewed methods, the NFPA (National Fire Protection Association) hazard ratings, along with globally recognized hazard pictograms and safety statements, were sourced from PubChem. These indicators were used to systematically assign hazard scores, reflecting the toxicity, flammability, and environmental risks of the chemicals involved **[7][8]**.

2.2. Analytical GREEnness Metric (AGREE)

AGREE (Analytical GREEnness Evaluator) is an advanced tool designed to assess the environmental sustainability of

analytical techniques in direct accordance with the 12 principles of Green Analytical Chemistry (GAC). This tool operates via a graphical user interface (GUI) and delivers a quantitative score ranging from 0 to 1, where a score of 1 indicates maximum greenness and 0 reflects no compliance with green principles.

It generates a circular pictogram composed of 12 distinct, color-coded segments, with each segment reflecting one of the GAC principles. These include parameters such as sampling strategy, sample volume, number of procedural steps, degree of miniaturization, derivatization requirements, waste generation, multiplexing (number of analytes), energy efficiency, reagent safety, toxicity of chemicals, and analyst safety. Users must input these method-specific parameters into the software to generate a comprehensive and visually intuitive greenness profile. AGREE provides a simple yet powerful means to compare the environmental performance of various techniques, supporting the development of eco-conscious practices in pharmaceutical and chemical analysis **[6][8]**.

In this review, Environmental impact of the reported analytical techniques was analyzed through the AGREE evaluation tool, which is grounded in the 12 core principles of Green Analytical Chemistry (GAC). To enhance the precision of the greenness assessment, a modified approach was implemented for calculating total solvent waste associated with each method.

The procedure involved two primary stages:

I. Instrumental Solvent Waste Calculation: The volume of solvent used during chromatographic analysis was determined by multiplying the instrument flow rate (mL/min) by the run time (min) and further adjusting for the typical number of analyses performed (approximately 5 to 6 replicates).

II. Sample Preparation Solvent Consumption: The amount of solvent consumed during sample extraction, dilution, or treatment steps was estimated based on method-specific details.

The total solvent waste was then calculated by summing the solvent used in both the instrumental and preparatory phases, providing a more holistic and realistic measure of each method's ecological footprint.

Total Solvent Waste = (Flow rate × Runtime × No. of runs) + Solvent used in sample preparation

3. Documented Techniques for the Estimation of Amiodarone and its Combinations [9-30]

This review compiled research articles related to the Assessment of Amiodarone in

pharmaceutical formulations by systematically searching databases such as PubMed, Google Scholar, ScienceDirect, Scopus, Taylor & Francis, and other credible online scientific sources.

3.1. High Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) remains one of the extensively employed and dependable techniques for the quantification of pharmaceutical compounds. Its popularity stems from its excellent sensitivity, accuracy, and reproducibility. Among the various chromatographic approaches, Reversed-Phase Liquid Chromatography (RP-LC) is the most frequently used mode, characterized by a hydrophobic stationary phase and a hydrophilic mobile phase. This setup favors the retention of more hydrophobic analytes, making it particularly effective for separating complex mixtures of organic substances. In the context of Amiodarone analysis, researchers have explored a wide range of mobile phase compositions, often incorporating organic solvents such as methanol, acetonitrile (ACN), chloroform, and triethylamine (TEA), along with buffer systems like phosphate and acetate-based solutions, to achieve optimal resolution and peak performance **[9-22]**. Tomez Pérez-Ruiz *et al*. established a novel HPLC technique with chemiluminescent detection designed for concurrent quantification of Amiodarone and Desethylamiodarone in serum and pharmaceutical dosage forms. Chromatographic separation was carried out on a C18 column with a mobile phase consisting of methanol and ammonium sulfate buffer at pH 6.8. Detection was performed via post-column photolysis and $Ru(bpy)_3^{3+}$-based chemiluminescence. The method demonstrated excellent sensitivity, with LODs of 0.02 µg/mL for Amiodarone and 0.11 µg/mL for Desethylamiodarone, and was linear over a wide range **[10]**. In another study, D. Marchiset *et al*. **[12]** developed a study using HPLC coupled with mass spectrometry to identify and quantify Amiodarone and its primary metabolite N-mono desethylamiodarone in patient plasma after a single oral or intravenous dose. It evaluated the pharmacokinetics, reporting a Blood-plasma half-life of ~21 hours, oral bioavailability of ~60%, and higher metabolite accumulation after oral dosing. The findings highlighted significant metabolite buildup with repeated administration **[12]**. In another study, S. Karmarkar *et al*. developed a study focused on optimizing the USP HPLC based approach for detecting impurity profiling of Amiodarone, incorporating an L1 stationary phase column (ODS2,

4.6 × 150 mm, 5 µm) with UV absorbance at 240 nm. Initial issues with column variability and inconsistent retention of impurity D led to a Quality by Design (QbD)-based optimization using Fusion AE™ software. The improved method employed the Atlantis T3 column, which resolved lot-to-lot variability and stabilized impurity retention. The optimized method met USP 〈621〉 allowances and was fully validated for accuracy, precision, linearity, robustness, and specificity **[15]**. The table 3 enumerates the existing HPLC methodologies for the measurements of amiodarone, both as an isolated molecule and in combinations.

3.2. Liquid chromatography-Tandem mass spectrometry

A Liquid chromatography-Tandem mass spectrometry technique was developed by Pauline M. Lacrok *et al*. which constructs a validated LC method to estimate amiodarone HCl and 10 related impurities in both raw materials and tablet forms. The method uses a 3 µm Hypersil nitrile column, with a 1:1 acetonitrile–ammonium acetate buffer (pH 6.0) as the mobile phase, a 1 mL/min flow rate, and UV absorbance at 240 nm. The method detects impurities as low as 0.02%, and drug content was found to be ~100% in raw materials and 98.2–99.4% in tablets. LC-MS confirmed 3 known impurities, and cross-lab validation proved the method reliable **[23]**.

3.3. High Performance Thin Layer Chromatography

A High Performance Thin Layer Chromatography method was developed by Nguyen, K which delivers a study developed and validated quantitative HPTLC–densitometry methods for Amiodarone HCl and other drugs, based on a model that transitions existing qualitative TLC screening methods into robust, quantitative protocols. These methods used green solvents, silica gel F254 plates, and automated application and detection, providing cost-effective and eco-friendly alternatives for drug quality testing. The work also introduced new qualitative TLC methods for drugs lacking screening protocols, with open-access publication in the FDA Compendium **[24]**. In another study, Dr. Santhosh developed an HPTLC-based analytical procedure that was developed and proven suitable for detecting stability-related changes. for the quantification of Amiodarone Hydrochloride in both bulk drug and pharmaceutical formulations. Silica gel 60 F254 plates were employed as the stationary phase, using a mobile phase composed of ethyl acetate and methanol in a 9:1 (v/v) ratio, achieving an Rf value of 0.48 ± 2.05. Detection at 242 nm. The

method was established per ICH Q2(R1) guidelines and demonstrated good linearity (200–1200 ng/band, R^2 = 0.9976), along with acceptable sensitivity and recovery. The drug was also tested under forced degradation studies, validating its stability-indicating nature **[25]**.

3.4. Nonaqueous Electrokinetic Chromatography (NAEKC)

Mol, R. carried out a study applied nonaqueous electrokinetic chromatography (NAEKC) using anionic sulfated cyclodextrins for impurity profiling of Amiodarone stored for one year. Detection was performed via both UV and ESI-ion trap MS, revealing 12 impurities, including 3 known and 3 newly proposed structures. The method demonstrated improved selectivity and sensitivity, with LODs of 1 µg/mL (UV) and 15 ng/mL (MS), confirming its suitability for impurity analysis of poorly water-soluble drugs like Amiodarone **[26]**.

3.5. Micellar electrokinetic chromatography (MEKC)

Pérez-Ruiz, T developed a capillary electrophoresis (CE) method for the rapid and sensitive separation of Amiodarone and its primary metabolite in serum matrices. Separation was achieved using a 45 cm capillary, 15 mM ADA buffer (pH 7.5), 10 mM SDS, and 70% acetonitrile, with 25 kV voltage. Sample introduction was carried out using electrokinetic techniques combined with field-amplified stacking enhanced sensitivity, achieving an LOD of 6.46 ng/mL and precision of 3.7%, confirming the method's suitability for bioanalysis **[27]**.

3.6 Ultra Performance Liquid Chromatography-Mass spectrometry

A UPLC-MS/MS method was developed by Ying Zhang *et al*. established and validated to simultaneously quantify Amiodarone, Dronedarone, and their metabolites in rat plasma. Acetonitrile was used to precipitate proteins during sample preparation, prior to chromatographic separation on an Acquity BEH C18 column and detection using a Xevo TQ-S triple quadrupole MS system set to positive electrospray ionization. The method showed excellent linearity, sensitivity (LLOQs: 0.1–1.0 ng/mL), and precision (≤13.3%), with recoveries above 82% and no significant matrix effects. It was successfully applied to pharmacokinetic studies in Sprague-Dawley rats **[28]**. In another method, Ramzia I. El-Bagary *et al*. proposed and validated two analytical chromatographic methods to quantify Amiodarone (AMI) and Dronedarone (DRO) simultaneously., especially to detect potential counterfeit substitution of AMI for the costlier DRO. The first method used RP-HPLC with a BDS Hypersil C18

column and methanol–phosphate buffer (90:10) at 2 mL/min, where detection was performed at 254 nm using a UV detector. The subsequent method used RP-UHPLC on an Acclaim RSLC 120 C18 column, with a methanol–buffer (95:5) mobile phase at 1 mL/min, also with UV detection at a wavelength of 254 nm. Both methods were linear (5–80 µg/mL), accurate, precise, and effective for ensuring the quality of pharmaceutical preparations **[29]**.

3.7. UV-Vis spectrophotometry

UV-Visible spectrophotometry remains an essential and widely utilized technique as it offers Practicality, affordability, and consistent performance, it is widely employed for quantifying pharmaceutical substances. It plays a critical role in routine drug assays and can also be combined with advanced methods like HPLC to enhance analytical performance. In the case of Amiodarone, UV methods have been developed using organic solvents notably methanol, acetonitrile, and chloroform. The drug exhibits significant absorbance at multiple wavelengths, typically around 303, 353, 400, 535, and 570 nm, depending on solvent and pH conditions. In this review, the reported UV methods for Amiodarone have been evaluated using two greenness assessment tools GAPI and AGREE to provide insight into their environmental sustainability and analytical efficiency **[9, 30, 31]**.

4. DISCUSSION

The comprehensive evaluation of analytical approaches for the quantification of Amiodarone reveals numerous types of instrumental techniques, each offering distinct advantages in terms of sensitivity, selectivity, analytical scope, and eco-friendliness. Among the reviewed literature, HPLC emerged as the most widely adopted technique, appearing in over 60% of the studies, owing to its reliability, precision, and compatibility with routine quality control. However, the increased use of solvents such as methanol, acetonitrile, and phosphate buffers in HPLC poses considerable environmental and occupational safety concerns.

In contrast, UV-Vis spectrophotometry, though limited by lower selectivity and higher interference susceptibility, offers a greener and simpler alternative, particularly in early-phase formulation screening or content uniformity testing. Its minimal solvent and energy requirements contribute to significantly lower environmental impact, as also reflected in higher AGREE and GAPI scores compared to more complex techniques.

High-Performance Thin Layer Chromatography (HPTLC) and Capillary Electrophoresis (CE) methods

demonstrated a favorable balance between sensitivity and greenness. These methods consumed relatively less solvent per run and involved reduced sample preparation time. Importantly, the HPTLC methods utilized greener mobile phases and silica-based plates, aligning closely with green analytical chemistry principles.

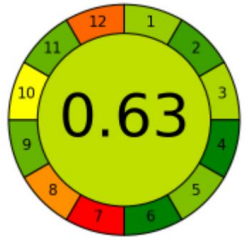
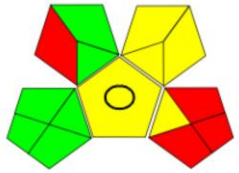
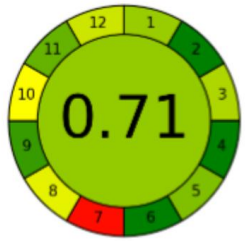
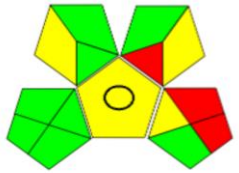
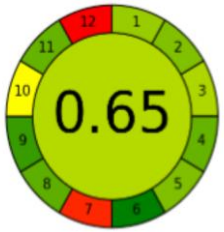
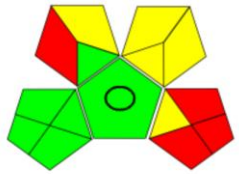
Advanced techniques such as UPLC-MS/MS and LC-MS provided superior analytical performance, including high sensitivity, rapid run times, and metabolite profiling capability. However, these techniques typically involve higher solvent consumption, energy use, and generation of hazardous waste. Despite their critical role in bioanalytical and pharmacokinetic studies, they yielded comparatively lower greenness scores in AGREE and GAPI evaluations.

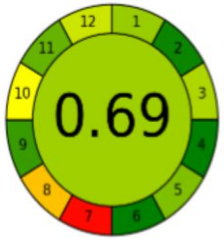
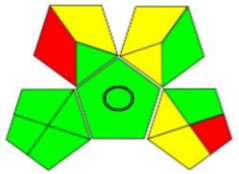
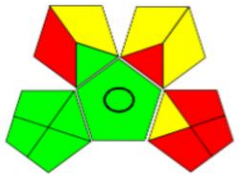
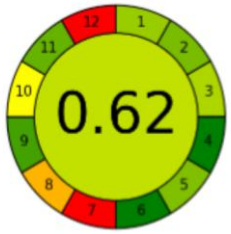
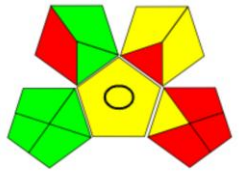
Notably, the application of NAEKC for impurity profiling of aged Amiodarone provided an innovative and green approach for analyzing poorly water-soluble drugs. It demonstrated enhanced selectivity using cyclodextrin-based modifiers and minimized sample and solvent requirements, making it an ideal candidate for sustainable analytical development.

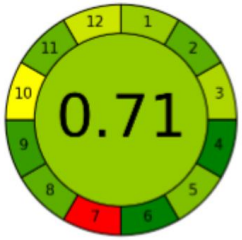
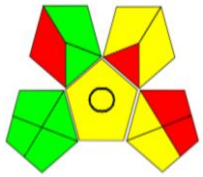
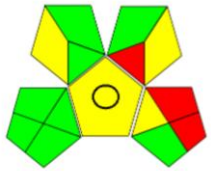
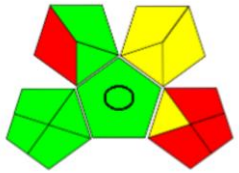
The greenness evaluation using GAPI and AGREE tools allowed for a structured and transparent assessment of each method's environmental burden. Methods with minimal sample preparation, reduced solvent consumption, and fewer derivatization steps scored significantly higher. For example, UV spectrophotometric methods and CE-based assays consistently ranked greener, while HPLC and UPLC-based techniques, though analytically robust, often scored lower due to higher waste generation and reagent toxicity.

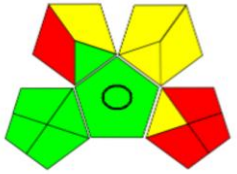
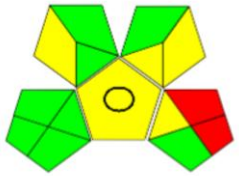
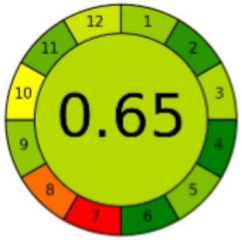
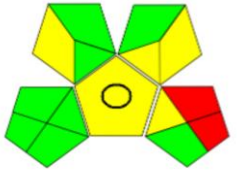
This analysis underscores the critical importance of balancing analytical efficiency with environmental sustainability. While high-end techniques are indispensable for regulatory compliance and complex matrices, there is a clear opportunity to optimize classical techniques and apply green chemistry principles especially in early-stage drug development and routine QC workflows.

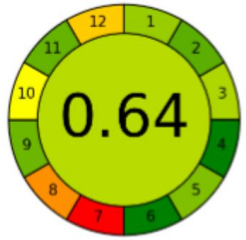
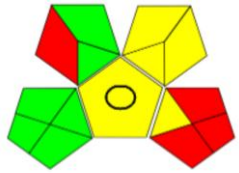
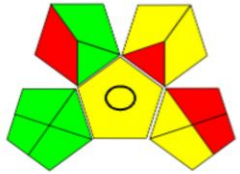
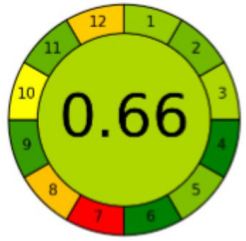
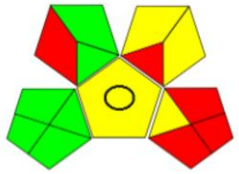
Table 3: Comparative overview of analytical methodologies for Amiodarone estimation across various matrices, integrating greenness metrics (GAPI & AGREE) for ecological profiling

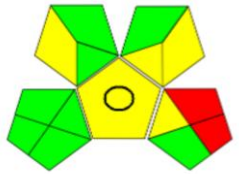
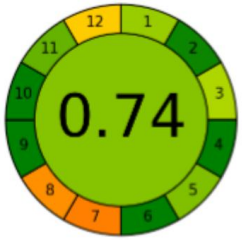
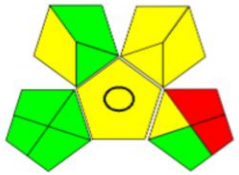
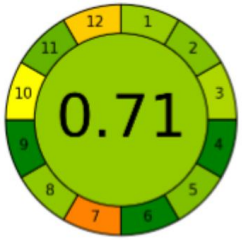
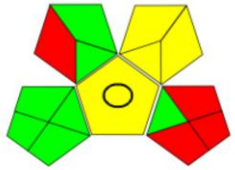
S. No.	Matrix	Techniques	Mobile phase / Solvent	λmax (nm)	Flow Rate (mL/min)	AGREE	GAPI	Authors
1	Pharmaceutical formulations	HPLC	Methanol : Tetrahydrofuran : TEA (48:34:18)	256	1.2			An na M. Di Pietra *et al.* [9]
2	Pharmaceutical formulations	HPLC-UV	Methanol : Ammonium Sulfate (92:8)	UV & Chemiluminescent detection	0.8			Tomez Pérez-Ruiz *et al.* [10]
3	Human plasma, myocardium	HPLC-UV	Acetonitrile : Isopropanol : Water : Ammonia (80:10:10:0.025)	254	1			Robert W. Bolderman *et al.* [11]

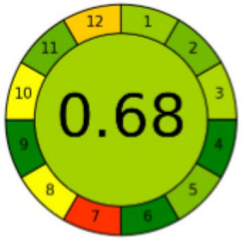
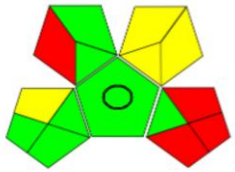
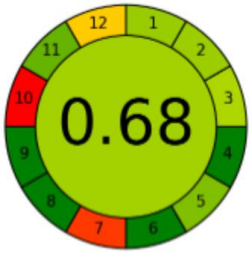
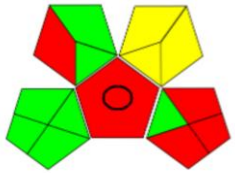
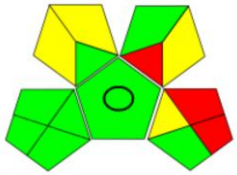
4	Human plasma	HPLC-UV & MS	Chloroform : Ethanol : Ammonia (99.71:0.25:0.04 v/v/v)	254	2			D. Marchiset *et al.* [12]
5	Plasma & heart tissue	RP-HPLC-UV	Acetonitrile : Methanol : 10 mM Sodium phosphate buffer (70:10:20, pH 4)	242	1			A. A. Al-Dhawailie [13]
6	Bulk & dosage forms	RP-HPLC	0.5% v/v TEA buffer (pH 6.5): Acetonitrile (25:75)	240	1			Vemugunta Ramakrishna *et al.* [14]

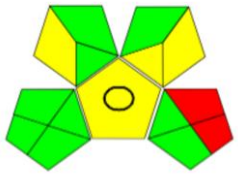
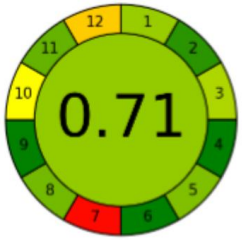
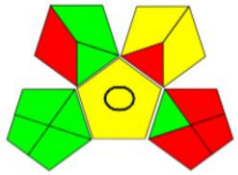
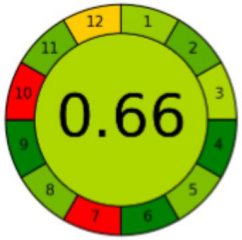
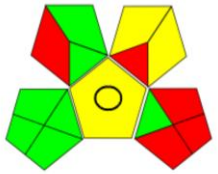
7	Drug formulation	RP-HPLC	Acetate Buffer : ACN : Methanol (20:46:34)	240	1			S. Karmarkar *et al.* [15]
8	Pharmaceutical dosage forms	RP-HPLC	Methanol : Water : Acetic acid (95:4:1)	242	1.5			N. Thyagarajapuram *et al.* [16]
9	Amiodarone HCl Injection	RP-HPLC	Mobile Phase A: 15 mM potassium phosphate monobasic + 30 mM triethylamine (pH 2.5); Mobile Phase B: Acetonitrile : Methanol (1:1 v/v)	240	1			Matthew J. Christopherson *et al.* [17]

10	**Plasma**	**RP-HPLC**	**Acetonitrile : Water : Propylamine (56:44:0.01 v/v/v)**	**254**	**1**			**M. De Smet *et al.* [18]**
11	**Amiodarone HCl API**	**RP-HPLC**	**Methanol : Water : Glacial Acetic Acid (75:24:1)**	**254**	**1**			**M. A. Khan *et al.* [19]**
12	**Paediatric capsules (10, 60, 100 mg)**	**RP-HPLC**	**0.01 M phosphate buffer : Methanol (17:83 v/v)**	**242**	**1**			**L. Rughoo *et al.* [20]**

13	**Tablet formulation**	**RP-HPLC**	**Buffer pH 5.0 : Methanol : Acetonitrile (30:30:40, v/v/v)**	**240**	**1**			**Fuad Al-Rimawi *et al.* [21]**
14	**Inclusion complex (HP-β-CD) & Cordarone® tablets**	**RP-HPLC-UV**	**Formic acid 0.5% in phosphate buffer (pH 7.6) : Methanol (25:75 v/v)**	**254**	**0.7**			**Andreea Bosînceanu *et al.* [22]**
15	**Raw materials & Tablets**	**LC-UV & LC-MS/MS**	**Acetonitrile : 0.1 M Ammonium acetate buffer (1:1)**	**240**	**1**			**Pauline M. Lacrok *et al.* [23]**

16	**Pharmaceutical formulation**	**HPTLC–Densitometry**	**EtOH & MeOH**	**254**	**NA**			**K. Nguyen *et al.* [24]**
17	**Bulk & Tablet dosage forms**	**HPTLC–Densitometry**	**Ethyl acetate : Methanol (9:1 v/v)**	**242**	**NA**			**Dr. Santosh V. Gandhi *et al.* [25]**
18	**Bulk drug**	**NAEKC-UV & MS**	**Acetonitrile–Water (75:25, v/v) with 0.1% formic acid or acetic acid**	**MS detection / CE migration time and Pressure-based**	**NA (CE system)**			**Roelof Mol *et al.* [26]**

19	**Serum**	**MEKC (CE)**	**15 mM ADA buffer (pH 7.5), 10 mM SDS, 70% v/v acetonitrile**	**DAD Detection**	**Capillary flow (pressure-driven)**			**T. Pérez-Ruiz *et al.* [27]**
20	**Rat plasma**	**UPLC-MS/MS**	**Acetonitrile : 0.1% formic acid**	**MS only**	**0.3**			**Ying Zhang *et al.* [28]**
21	**Human plasma**	**UPLC-MS/MS**	**Tea + Methanol (10:90)**	**254**	**2**			**Ramzia I. El-Bagary *et al.* [29]**

22	**Pharmaceutical formulations**	**UV Spectrophotometer**	**Methanol**	**303**	**NA**			**Anna M. Di Pietra *et al.* [9]**
23	**Commercial dosage forms**	**UV-Spectrophotometry (Method A & B)**	**Method A: Methanol-Acetone with N-bromosuccinimide; Method B: Chloroform with Bromothymol Blue in pH 2.32 buffer**	**Method A: 353; Method B: 400**	**NA**			**Nafisur Rahman *et al.* [30]**
24	**Commercial dosage forms**	**UV-Spectrophotometry (Method A & B)**	**Method A: Dioxan-Dichloromethane with p-Chloranilic Acid; Method B: Acetonitrile with DDQ**	**Method A: 535; Method B: 570**	**NA**			**Nafisur Rahman *et al.* [31]**

5. CONCLUSION

A total of 24 analytical techniques for estimating Amiodarone were systematically reviewed in this study, covering a wide range of techniques including HPLC, UV, HPTLC, UPLC-MS, LC-MS, CE, and NAEKC. While HPLC remains the most widely used method, techniques like UV and HPTLC offered greener alternatives with acceptable analytical performance. The GAPI and AGREE assessments highlighted that several methods can be optimized for eco-friendliness without compromising reliability. This study supports the integration of green chemistry principles in routine pharmaceutical analysis and method development for Amiodarone.

6. FUTURE SCOPE

Future studies should focus on the development of multi-analyte green methods that allow simultaneous quantification of Amiodarone with co-administered cardiovascular drugs. There is also scope for incorporating miniaturized, solvent-free techniques to reduce reagent usage and environmental impact. The integration of Quality by Design (QbD) principles and AI-assisted tools can further optimize method development from both analytical and ecological perspectives. Additionally, expanding greenness evaluations using tools like the Analytical Eco-Scale and extending such assessments to bioanalytical methods will help build a more sustainable framework for pharmaceutical analysis.

Author contributions

Suleman Basha H: Conceptualization, Data curation, Investigation, Visualization, Original draft, Writing and editing review.

Dr. Kavitha Jayaseelan: Project administration, editing, and supervision.

Dr. Manikandan: Project administration, editing.

Conflict of interest

The authors declare no conflicts of interest.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgment

The authors are thankful to the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, for providing the facilities to carry out the work successfully.

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