INTERNATIONAL JOURNAL OF HEALTH & MEDICAL RESEARCH
ISSN(print): 2833-213X, ISSN(online): 2833-2148
Volume 05 Issue 05 May 2026
DOI : 10.58806/ijhmr.2026.v5i5n10
Page No. 406-412

2,4-Dinitrophenylhydrazine used for Spectrophotometric Determination of Amiodarone in Pure Form and Pharmaceutical Preparations

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ABSTRACT: For the measurement of amiodarone in its pure form and in pharmaceutical dose preparations, a straightforward, quick, and extremely sensitive spectrophotometric approach was created and verified. The suggested analytical method is based on an oxidative coupling reaction in a very alkaline media between amiodarone and oxidized 2,4-dinitrophenylhydrazine (2,4-DNPH), which produces a persistent yellowish-green chromogenic product. Spectrophotometric measurements of the generated color's intensity were made against a reagent blank at a maximum absorption wavelength of 622 nm. Reagent quantities, alkaline medium type and concentration, oxidant concentration, reaction time, temperature, and addition sequence were all carefully adjusted to maximize sensitivity and repeatability. With a high molar absorptivity of 2.32×10^4 L·mol^{-1}·cm^{-1}, the technique demonstrated good sensitivity under ideal conditions and obeyed Beer–Lambert's law over a concentration range of 1.5–30 µg·L^{-1}. It was discovered that the limits of detection (LOD) and quantification (LOQ) were 1.11 and 2.32 µg·L^{-1}, respectively. Recovery values of 99.87% and a relative standard deviation (RSD) of 1.75% attested to the developed method's exceptional accuracy and precision. A stable reaction product was formed, as evidenced by the stoichiometric ratio of 1:1 between amiodarone and 2,4-DNPH. The method's selectivity was confirmed by interference studies, which showed that typical pharmaceutical excipients and surfactants such glucose, lactose, starch, and acacia did not significantly alter the result. By determining the amount of amiodarone in commercial tablet formulations, the suggested method's applicability was effectively confirmed, producing dependable and satisfactory results. The suggested spectrophotometric method is a useful and efficient substitute for more intricate and costly analytical techniques for routine quality control analysis of amiodarone in pharmaceutical laboratories because of its simplicity, affordability, quick response, and high sensitivity.

KEYWORDS: Amiodarone; Spectrophotometry; Oxidative coupling; Accuracy; Precision.

INTRODUCTION

A powerful class III antiarrhythmic drug, amiodarone is frequently used to prevent and treat a variety of cardiac dysrhythmias(1). It was initially produced in 1962 and used in clinical settings to treat angina pectoris, or chest pain(2). It was reintroduced in 1974 after demonstrating its remarkable effectiveness in treating life-threatening arrhythmias, despite being temporarily removed from numerous markets in the mid-1970s due to worries about its side-effect profile(3). It is still one of the most commonly prescribed medications for cardiac rhythm problems today(4). Amiodarone ($C_{25}H_{29}I_2NO_3$) is a benzofuran derivatives with a high iodine concentration that weighs about 645.31 g/mole(2). Because of its high lipophilicity and wide tissue distribution, the medication has a slow beginning of action when taken orally, frequently taking several weeks to produce therapeutic effects(5). In the liver, it is extensively metabolized, mostly by the cytochrome P450 3A4 (CYP3A4) enzyme system(6). Because amiodarone can impede the clearance of other medications, such warfarin, increasing the risk of toxicity and anticoagulant effects, this metabolic route is clinically significant(7).

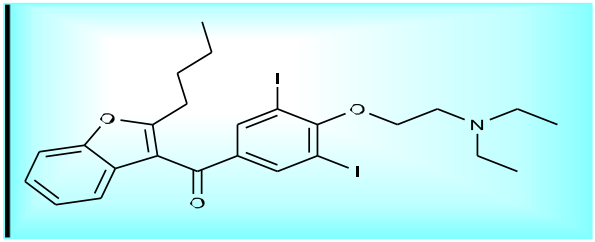


Figure (1): Amiodarone (645.31 g/mole) C25H29I2NO3 (8).

2,4-Dinitrophenylhydrazine used for Spectrophotometric Determination of Amiodarone in Pure Form and Pharmaceutical Preparations

Amiodarone has therapeutic benefits, but long-term usage is linked to a number of side effects. Fatigue, nausea, tremors, and constipation are common side effects(9); pulmonary toxicity and thyroid dysfunction are more serious issues. Amiodarone's iodine content and structural resemblance to thyroid hormones frequently cause patients' thyroid function tests to change(10). Additionally, the medication may result in hepatic problems, which can range from asymptomatic increases in liver enzymes to uncommon instances of cirrhosis or hepatitis(11). Therefore, accurate drug monitoring in pharmaceutical preparations is critical to patient safety. This study's main goal is to develop a novel spectrophotometric technique for amiodarone quantification that is affordable and simple to use in conventional labs(12). Despite the availability of sophisticated chromatographic methods, spectrophotometry is still the method of choice for pharmaceutical analysis because of its affordability and ease of use(13). The goal of this work is to maximize the oxidative coupling process between 2,4-DNPH and amiodarone(14). The study intends to develop a validated analytical instrument for the precise measurement of amiodarone in both its pure state and commercial tablet forms by examining a number of factors, including reagent volume, temperature, reaction duration, and the impact of alkaline media.

EXPERIMENTAL

Preparation solutions

1.2,4-dinitrophenyl hydrazine (2,4-DNPH) ($4x10^{-3}$ mole. L^{-1}) prepared by dissolve 0.08g in 3mL of concentration H_2SO_4 and completed to 100 mL by distilled water.

2. Potassium periodate (KIO4) (0.1 mole. L^{-1}) prepared when dissolving 2.3g of KIO_4 in 100 mL with distilled water.

3.Sodium hydroxide 1 mole. L^{-1} prepared by dissolve 4g in 100 mL of distilled

4.1000 µg. mL^{-1} from standard solution of amidarone prepared by dissolving 0.1g and diluted to 100 mL distilled water.

5.Interference solution 1000 µg. L^{-1}, 0.1g for each foreign compound was dissolved in distills water there completed to 100mL in volumetric flask.

RESULTS AND DISCUSSION

Absorption spectrum of product

A 1ml of 2,4-DNPH is added to 1mL of KIO_4 0.1 mole. L^{-1} after that 2mL of amiodarone solution 100 ppm was added in the presence of basic medium by using 0.5 mL of 1 mole. L^{-1}NaOH then diluted by distilled water to 25 mL volumetric flask. The reaction between oxidized of 2,4-DNPH with amiodarone in alkaline medium give colored product yellow – green, under quickly test shows maximum absorption λmax at 622 nm against to blank reagent figure (2).

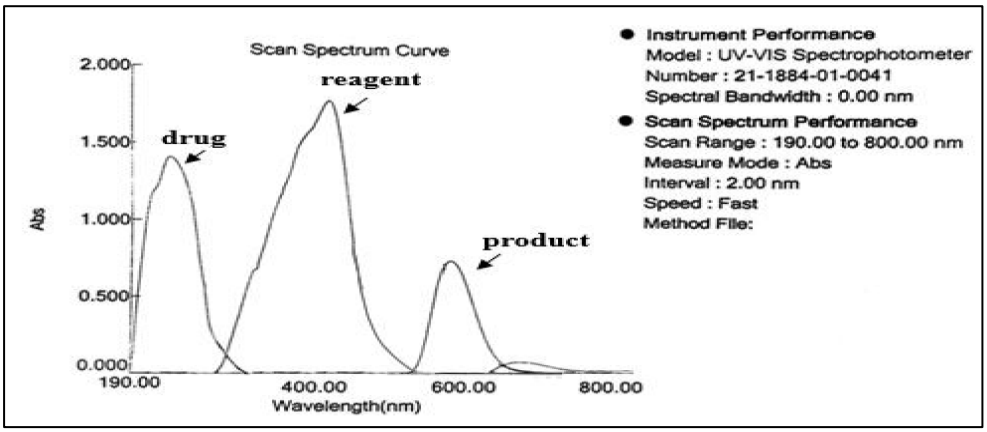
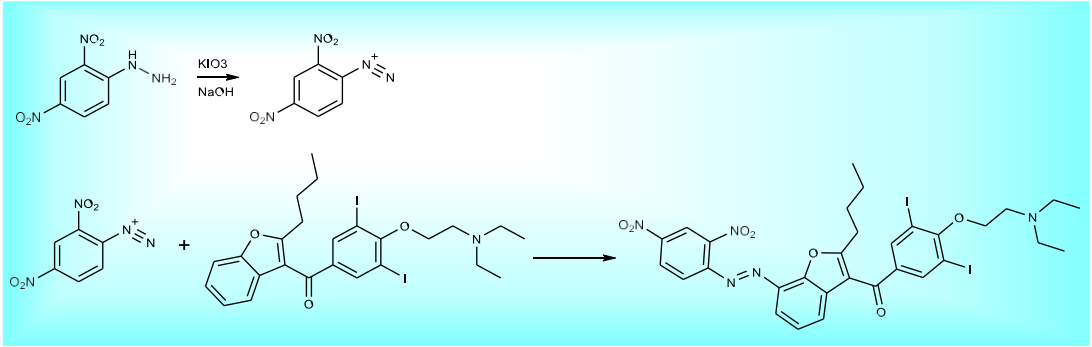


Figure (2): Absorption spectra of drug, reagent and product. The reaction can be represented in scheme (1) below

Scheme (1) Preparation of product



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OPTIMUM CONDITIONS

A .01mL of amiodarone solution (100 µg. mL^{-1}), 1.5mL of 2,4-DNPHZ $4x10^{-3}$ mole. mL^{1} and 1.0 mL of KIO_4 in alkaline medium (0.7mL) of 1 mole. L^{-1} NaOH put in 10.0 mL volumetric flask then completed to 10.0 mL, were studied for establish the optimum conditions.

SELECTION THE VOLUME OF THE 2,4-DNPHZ REAGENT

Different volume of 2,4-DNPHZ from (0.5-3.5) mL used, then measured absorbance of the formed product agonist reference blank, from table (1) found the 1.5 ml of 2,4-DNPHZ give higher absorbance, therefore chosen 1.5mL of $4x10^{-3}$ mole. L^{-1} used in all subsequent measurements.

Table (1): Different volume of 2, 4-DNPHZ $4x10^{-3}$M

mL of 2,4-DNPHZ	0.5	1	1.5	2	2.5	3	3.5	4
Absorbance	0.358	0.511	0.881	0.851	0.844	0.843	0.819	0.833

Effect of the sodium hydroxide

Different volume of 1.0 mole. L^{-1} of NaOH from (0.1-1.0) mL used, from table (2) found the 0.7mL of NaOH give the highest absorbance. The 0.7 mL of NaOH was used in all the study.

Table (2): Different volume of NaOH (0.1M)

mL of NaOH 1.0 mole. L^{-1}	0.1	0.3	0.5	0.7	1
Absorbance	0.257	0.669	0.793	0.866	0.811

Effect of Potassium periodate (KIO_4)

In this study used a different volume (0.5-3.5) ml of 0.1 mole. $L^{-1}KIO_4$, from table 3 found 2.0 mL gave the highest absorbance, therefore 1.0 mL 0f KIO_4 used in all the study.

Table (3): Different volume of KIO_4 (0.1M)

mL of KIO_4 0.1 mole. L^{-1}	0.5	1	1.5	2	2.5	3
Abs.	0.525	0.892	0.831	0.809	0.783	0.775

Effect of time on reaction

The absorbance of reaction was recorded with different time from (5-60) min., from table 4 the 15 min. can be the beast time to completed of oxidative coupling reaction.

Table (4): Effect of time on reaction

Time Min.	5	10	15	20	25	30	40	50
Abs.	0.621	0.857	0.861	0.860	0.860	0.860	0.860	0.860

Effect of Temperature

The different of temp. From (5-60) C^0on the absorbance of the color product was studied. From table 5 the optimum temp. Is (20-30) C^0, because it gives the best absorbance, therefore it used of the subsequent experiments.

Table (5): Effect of temperature

Temp.C^0	15	20	25	30	40	50	60
Abs.	0.441	0.699	0.802	0.754	0.498	0.311	0.185

Effect of the order of additions

Table (6) indicate the addition (2, 4-DNPHZ+ KIO4+amiodarone + OH^-) is the beast order because it give the highest absorption of the color product, so it used in all study.

Table (6) Order of additions

Order number	Order of addition	Abs.
1	KIO_4 +2,4DNPH+ amiodarone +OH^-	0.698
2	2,4DNPH+KIO_4+ amiodarone +OH^-	0.852
3	amiodarone +OH-+2,4DNPH+ KIO_4	0.678
4	amiodarone + KIO_4+2,4DNPH+ OH^-	0.385

2,4-Dinitrophenylhydrazine used for Spectrophotometric Determination of Amiodarone in Pure Form and Pharmaceutical Preparations

Chosen the Base

Sodium hydroxide, lithium hydroxide and potassium hydroxide were examined for sensitivity and reproducibility for spectrophotometric results. Table (7) indicate the NaOH give a good absorption value at the wave length 622 nm, therefore it use in all study.

Table (7) Effect of the base

Alkaline medium 1.0 mole. L^{-1}	LiOH	NaOH	KOH
Abs.	0.632	0.855	Turbid

CONSTRUCTION OF CALIBRATION CURVES AND ANALYTICAL DATE

After preparing a range of concentration from (1-50) µg. mL-1 of amiodarone and form color product under optimum condition and measure the absorption at 622nm against the blank reagent. Figure (3) indicate the obeyed of beers law from 1.5 – 30 µg. $mL^{-1}$with molar absorptivity $2.32x10^4$.

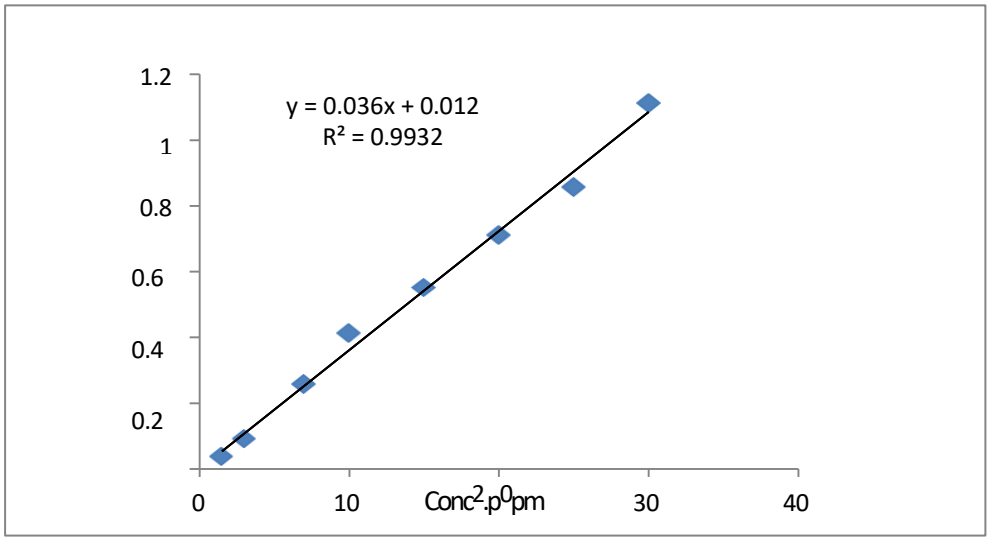

Figure (3): Calibration curve of determination of Amiodarone under optimum condition

Table (8) including many métiers and statistical data are measured such as LOD, LOQ, recovery and relative standard deviation RSD%.

Table (8): Statistical data of determination of amiodarone.

Parameter	Value
λmax(nm)	622
color	Yellowish – green
Linearity range µg·mL^{-1}	1.5-30
Regression equation	Y=0.036 X + 0.012
Slope	0.036
Correlation Coefficient	0.9932
Molar absorptivity $L.mol^{-1}.cm^{-1}$ [19]	$2.32*10^4$
Sandells sensitivity $mg.cm^{-2}$ [20]	0.027
Limit of Detection µg·mL^{-1}[21]	1.11
Quantitative limit µg·mL^{-1}[22]	2.32
Relative standard deviation %	1.75
Recovery%	99.87
Stoichiometry	1:1

Precision and Accuracy

In order to determination the precision and accuracy of proposed method were studied by calculating the value of relative standard deviation (R.S.D%) and relative error (E%) to the three different concentration of amiodarone drug. Acceptable values from table (9) for precision and accuracy

Table (9) Precision and accuracy for determination of amiodarone (µg. L^{-1})

Taken	Found	E%	RSD%
5	4.895	0.021	1.787
10	10.113	- 0.011	2.045
20	20.358	- 0.017	1.442

Stoichiometry(15)

Jobs and mole ratio method used to determination of the ratio of 0. 01mole.L^{-1} amiodarone and 0.01 mole. L^{-1}2,4-DNPH reagent figure (4) and (5) illustrates the ratio of amiodarone and reagent 1:1

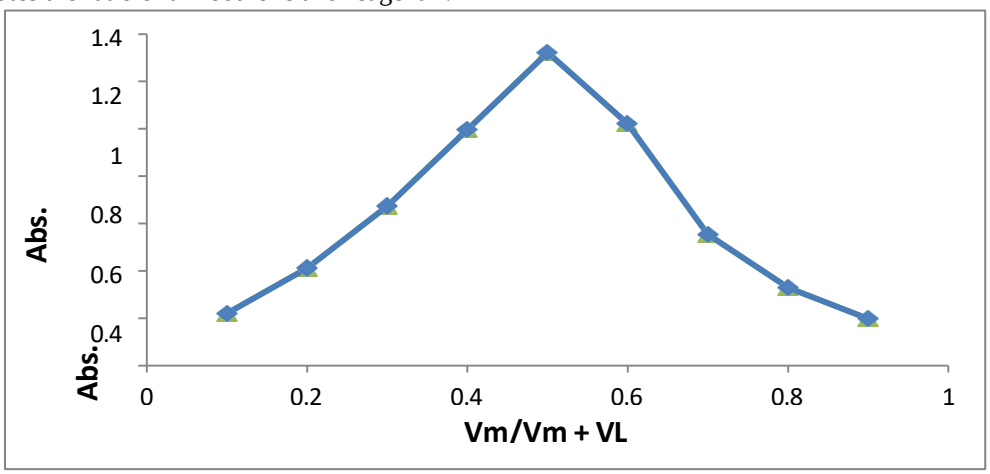

Figure (4): Jobs Method

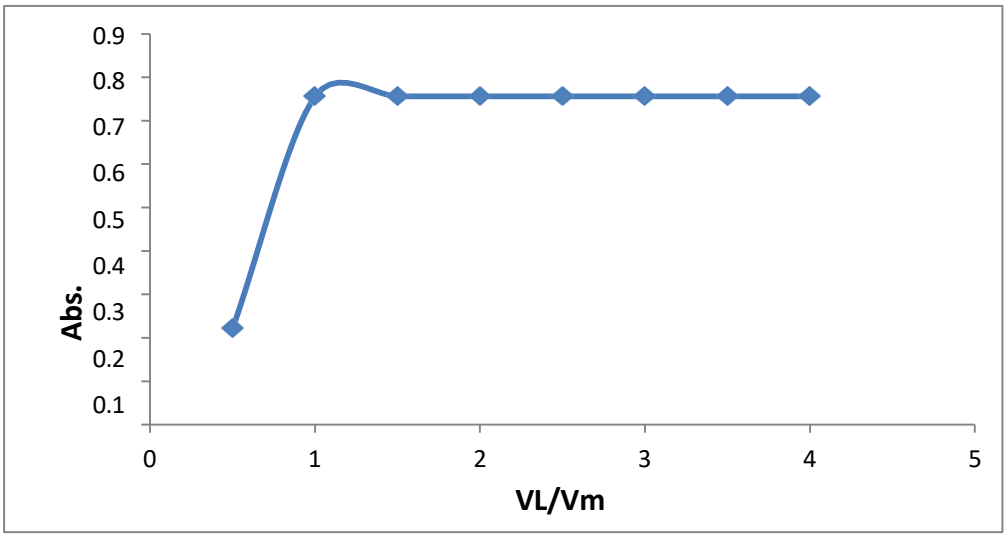

Figure (5): Mole ratio

Stability constant (16)

In order to determination the stability constant we must measure the dissociation degree α and stability constant (K_{st}) values, table (10) summarized the values of stability constant.

Table (10): Stability constant of Amiodarone with 2,4-DNPH

Drug	Conc. on of drug (mole. L^{-1})	Absorbance as a functional of quantitative conc.	Absorbance with increase of 2,4DPNH	Degree of dissociation (e)	Kst. $L.mol^{-1}$
Amiodarone		1.784	1.758		

Interference (surfactants effect) (17)

Table (11) illustrates the effects different surfactants such as lactose, starch, glucose and acacia, from the results show no discernible interaction with above surfactants, therefore suggested technique may be utilized for the pharmaceutical preparation.

Table (11): Surfactants on amiodarone

Surfactants	Conc. of surfactants	Taken	Found	E%	Re%
Glucose	1000	10	10.12	-0.012	100.012
Starch	1000	10	10.04	-0.4	100.4
Acacia	1000	10	9.89	0.011	99.989
Lactose	1000	10	10.15	-0.015	100.015

Applications

Proposed method applied and use amiodarone tablet to determine the amiodarone table (12) illustrates the results in the suggested method are given were satisfactory.

Table (12) Application of the determination of amiodarone in the tablet

Amiodarone present µg. L^{-1}	Amiodarone measure µg. L^{-1}	E%	RSD%	Recovery%
5	5.08	0.016	2.02	99.984
10	10.2	0.020	1.95	99.980
15	15.08	0.005	2.81	99.995

CONCLUSIONS

The following inferences can be made in light of the experimental findings and statistical analysis, Performance and Sensitivity, with a LOD of 1.11 µg. L-1, the discovered oxidative coupling reaction involving amiodarone and 2,4-DNPH demonstrated to be a very sensitive approach that made it possible to detect low drug concentrations. Stability in Analysis, over a concentration range of 1.5–30µg. L-1, the yellowish-green product generated in an alkaline medium exhibited exceptional stability and a high absorption signal at 622 nm, strictly according to Beer's law. Accuracy & Precision, the method's excellent recovery percentages (99.87%) and low RSD% (1.75%) attest to its high accuracy and reproducibility, making it appropriate for accurate pharmaceutical analysis. Selectivity, the method's strong selectivity for the ingredient that is active in complex formulations was demonstrated by the lack of significant interaction from popular excipients and surfactants including lactose, glucose, and starch. Practical Applicability in general, the suggested spectrophotometric method is quick, easy, and affordable. For the routine examination of amiodarone in its natural form and pharmaceutical tablets, it offers a viable substitute for more costly and time-consuming instrumental approaches.

REFERENCE

1) El Sheikh R, Gouda AA, Ghazy AA, Jumaa NM, Elsaify NE, Soliman NS, et al. Development and validation of spectrophotometric methods for estimation of arrhythmias drug-dronedarone hydrochloride in pure and dosage forms. Talanta Open. 2024;10:100355.
2) Kadhum MA, Hadwan MH. Simplified and economic measurement of glyoxalase I activity using 2, 4-dinitrophenylhydrazine: A valuable tool for researchers. Biology Methods and Protocols. 2025;10(1):bpaf013.
3) Palacios-López DL, Prieto-Blanco MC, Moliner-Martínez Y, Campins-Falco P. Carbonyl compounds responsive 2, 4-dinitrophenylhydrazine doped polydimethylsiloxane based membrane as alternative derivatizing strategy prior to in-tube solid phase microextraction coupled with capillary liquid chromatography analysis. Journal of Chromatography A. 2025;1740:465595.
4) Vinel PK, Grobovoy S, Sinitskii A, Kolesnikov O. Modification of a spectrophotometric method for assessment of monoamine oxidase activity with 2, 4-dinitrophenylhydrazine as a derivatizing reagent. Analytical Biochemistry. 2021;629:114294.
5) Nayif SM, Mohammed DH. Spectrophotometric Determination of Isoniazid in Pharmaceutical Preparations Using Oxidative Coupling Reaction with 2, 4-dinitrophenylhydrazine. Rafidain Journal of Science. 2023;32(1):149-60.
6) Esteves F, Rueff J, Kranendonk M. The central role of cytochrome P450 in xenobiotic metabolism—a brief review on a fascinating enzyme family. Journal of xenobiotics. 2021;11(3):94-114.
7) Zhang RX, Dong K, Wang Z, Miao R, Lu W, Wu XY. Nanoparticulate drug delivery strategies to address intestinal cytochrome P450 CYP3A4 metabolism towards personalized medicine. Pharmaceutics. 2021;13(8):1261.
8) Yao X, Gao S, Wang J, Li Z, Huang J, Wang Y, et al. Structural basis for the severe adverse interaction of sofosbuvir and amiodarone on L-type Cav channels. Cell. 2022;185(25):4801-10. e13.
9) Șorodoc V, Indrei L, Dobroghii C, Asaftei A, Ceasovschih A, Constantin M, et al. Amiodarone therapy: Updated practical insights. Journal of Clinical Medicine. 2024;13(20):6094.
10) CHANDANA J, MEGHANA GS, AASHRITHA B, GANESH B, RAO TR. A REVIEW ON CLINICAL ASPECTS OF AMIODARONE. 2023.

11) Castelino RD. Utilization Pattern and Adverse Effects of Antiarrhythmic Drugs and Role of Clinical Pharmacist Thereon: Sultan Qaboos University (Oman); 2022.
12) Lee PY, Bello J, Yarmohammadi H. Arrhythmogenic and Side Effects of Antiarrhythmic Medications. Cardiac Electrophysiology Clinics. 2025.
13) Cini N, Gölcü A. Spectrophotometric methodologies applied for determination of pharmaceuticals. Current Analytical Chemistry. 2021;17(8):1141-68.
14) Ahmed R. High-Performance Liquid Chromatography (HPLC): Principles, Applications, Versatality, Efficiency, Innovation and Comparative Analysis in Modern Analytical Chemistry and In Pharmaceutical Sciences. 2024.
15) Xiao Q, Song L, Wang X, Xu H, He L, Li Q, et al. Highly efficient extraction of palladium (II) in nitric acid solution by a phenanthroline-derived diamide ligand. Separation and Purification Technology. 2022;280:119805.
16) Ogechi OV, Ikejiofor OA. A Review of the Essence of Stability Constants in the ThermodynamicAssessments of Chemical Compounds. Journal of Material Sciences & Manufacturing Research. 2023;4(3):1-8.
17) Salih BM, Ali AM, Bayati M. Determination of iron concentration in blood using a chemiluminescence method with a mobile phone-based detector. Anaesthesia, Pain & Intensive Care. 2025;29(8).