Green Oxidative Coupling Spectrophotometric Method for Amiodarone Determination in Pharmaceutical Formulations

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A simple, sensitive, and cost-effective spectrophotometric method based on the oxidative coupling reaction of amiodarone was reported for the determination of amiodarone in pure form and pharmaceutical formulations. The method is based on measuring at 622 nm a yellowish-green stable chromogenic product formed in the reaction between amiodarone and 2,4-dinitrophenylhydrazine in an alkaline medium, with potassium iodate as the oxidizing agent. The main experimental conditions, such as reagent concentration, oxidizing agent volume, reaction time, alkaline medium, and order of addition, were optimized for the method. Under the optimized parameters, the final product has a molar absorptivity of 2.32 × 10⁴ L mol⁻¹ cm⁻¹, and the method is linear over the concentration range of 1.5–30 μg mL⁻¹, with a correlation coefficient of 0.9932. The limits of detection and quantification are 1.11 μg mL⁻¹ and 2.32 μg mL⁻¹, respectively. The developed method was successfully applied to the analysis of pharmaceutical formulations purchased from local pharmacies and demonstrated high precision and accuracy, with a relative standard deviation of < 2% and an average recovery of 101.4%.

Keywords: amiodarone, spectrophotometry, oxidative coupling reaction, 2,4-dinitrophenylhydrazine, pharmaceutical analysis

Introduction

Amiodarone is a widely used antiarrhythmic drug for treating and preventing various cardiac arrhythmias, including ventricular and supraventricular dysrhythmias [1]. The drug was first synthesized in the early 1960s and initially used for angina pectoris [2]. However, its strong antiarrhythmic properties led to broad clinical adoption, despite concerns about adverse effects [3]. Amiodarone has a complex pharmacokinetic profile and long half-life, resulting in a delayed therapeutic effect after oral administration [4]. Common side effects include fatigue, nausea, tremor, and constipation [5]. In long-term use, it may result in more serious complications, such as pulmonary toxicity, thyroid dysfunction, hepatotoxicity, and peripheral neuropathy, and may affect thyroid function and hormone metabolism due to its iodine content [6]. Abnormal liver enzyme levels and rare hepatic complications, including hepatitis, have also been reported in patients receiving prolonged therapy [7–9]. Amiodarone is extensively metabolized in the liver by cytochrome P450 enzymes and has a very low total body clearance and an extremely long terminal elimination half-life of 20-100 days [10]. That's why, given its widespread use and the potential risks of side effects, developing simple, accurate, and sensitive analytical methods for its determination in pharmaceutical formulations is essential.

Various analytical methods have been reported for the determination of amiodarone and its major active metabolite, desethylamiodarone (DEA), including high-performance liquid chromatography [11–13], spectrophotometric procedures [14], and electrochemical techniques [15–17]. Such techniques are very effective, with a low limit of detection. However, many of them require costly equipment, complex sample preparation, or extended analysis times. Developing a simple visible spectrophotometric method offers an appealing alternative for routine pharmaceutical analysis. Rahman et al. developed and validated spectrophotometric procedures using N-bromosuccinimide and bromothymol blue for the determination of amiodarone in dosage forms [14]. El Sheikh et al. reported validated spectrophotometric methods for the determination of dronedarone hydrochloride, a structural analog of amiodarone belonging to the same class of antiarrhythmic agents. The proposed procedures were based on oxidation reactions coupled with spectrophotometric monitoring of chromogenic systems and demonstrated satisfactory analytical performance over the concentration range of 1.0–15.0 μg mL⁻¹ [18]. However, the development of alternative spectrophotometric methods using different chromogenic and coupling reagents remains of considerable interest for routine pharmaceutical quality control.

This study developed a simple and sensitive spectrophotometric method for determining amiodarone, based on its oxidative coupling reaction with 2,4-dinitrophenylhydrazine in an alkaline medium with potassium iodate. The method was optimized and successfully applied to the analysis of amiodarone in pharmaceutical preparations.

Experimental part

Chemicals and Reagents. 2,4-Dinitrophenylhydrazine (2,4-DNPH, ≥99%), potassium iodate (KIO_4, ≥99.5%), sodium hydroxide (NaOH, ≥98%), and sulfuric acid (H_2SO_4, 98%) were purchased from Merck (Germany). Pure amiodarone standard was supplied by the State Company for Drug Industries and Medical Appliances (SDI, Samarra, Iraq). All chemicals and reagents were used without further purification. Distilled water further purified on a Milli-Q system was used for the preparation of all solutions. Commercial pharmaceutical tablet formulations containing amiodarone were purchased from local pharmacies.

Apparatus. Absorbance measurements were performed using a Shimadzu UV–Vis 1700 double-beam spectrophotometer with 1 cm quartz cells. An analytical balance (ISO LAB, Taiwan) was used for weighing all chemicals and reagents. pH measurements were performed using a Hanna Instruments pH meter (Germany).

Preparation of solutions. A 2,4-Dinitrophenylhydrazine (2,4-DNPH) solution at a concentration of 4×10^{-3} mol L^{-1} was prepared by dissolving 0.08 g of the reagent in 3 mL of concentrated sulfuric acid and diluting to 100 mL with water. A stock solution containing 1000 μg mL^{-1} amiodarone was prepared by dissolving 0.1 g of pure amiodarone in distilled water and diluting to a final volume of 100 mL. Working solutions were prepared daily by dilution with distilled water.

General analytical procedure. To aliquots of amiodarone with appropriate concentrations, 1.5 mL of 4×10^{-3} mol L^{-1} 2,4-Dinitrophenylhydrazine solution and 1.0 mL of 0.1 mol L^{-1} potassium iodate were subsequently added. After that, 0.7 mL of 1.0 mol L^{-1} sodium hydroxide solution was added to create the alkaline medium. Then, the mixture was diluted to a final volume of 10 mL with distilled water and allowed to stand for 15 min at room temperature to ensure complete color development. The absorbance of the resulting yellowish-green chromogenic product was measured at 622 nm against a reagent blank prepared under identical conditions in the absence of amiodarone.

Results and Discussion

Absorption spectrum and reaction mechanism. The oxidative coupling reaction between amiodarone and 2,4-dinitrophenylhydrazine was studied in an alkaline medium with potassium iodate as the oxidant. Under the optimized conditions, the reaction produced a stable yellowish-green chromogenic product with maximum absorbance at 622 nm, while the reagent blank exhibited negligible absorbance at this wavelength. The absorption maximum in the visible region suggests that oxidative coupling has produced an extended conjugated chromophoric system. The proposed reaction mechanism involves the initial oxidation of 2,4-dinitrophenylhydrazine by potassium iodate in an alkaline medium, generating a reactive intermediate that couples with amiodarone. The following oxidative coupling produces a stable-colored product suitable for spectrophotometric quantification. Stoichiometric investigations using Job's continuous variation and mole ratio methods confirmed the formation of a 1:1 reaction product between amiodarone and 2,4-DNPH under the selected.

Optimization of experimental conditions

Effect of 2,4-Dinitrophenylhydrazine concentration. The effect of 2,4-DNPH concentration on the oxidative coupling reaction was evaluated by varying the reagent volume from 0.5 to 4.0 mL, with all other variables held constant. As shown in Fig. 1, absorbance increased with reagent volume up to 1.5 mL, indicating greater formation of the colored product. Beyond 1.5 mL, absorbance slightly decreased, likely due to excess reagent and partial self-association. Based on these results, 1.5 mL of 4×10^{-3} mol L^{-1} 2,4-DNPH was chosen as the optimal reagent volume for further analyses.

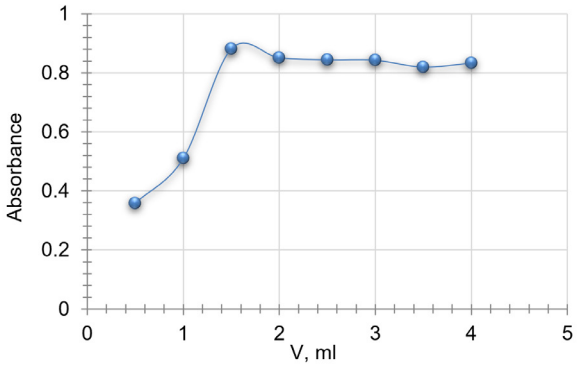

Fig. 1. Effect of 2,4-DNPH volume on the absorbance of the amiodarone–2,4-DNPH oxidative coupling product, $C_{2,4\text{-DNPH}} = 4 \times 10^{-3}$ mol L^{-1}.

Effect of potassium iodate concentration. The effect of oxidizing agent concentration was evaluated by varying the volume of 0.1 mol L^{-1} potassium iodate solution. The analytical signal increased with increasing oxidant volume, reaching a maximum absorbance at 1.0 mL of KIO_4, as shown in Fig. 2. Above this volume, absorbance gradually decreased, likely due to over-oxidation or decomposition of the chromogenic reaction product at higher oxidant concentrations. Therefore, 1.0 mL of potassium iodate solution was selected as the optimal volume of oxidizing agent.

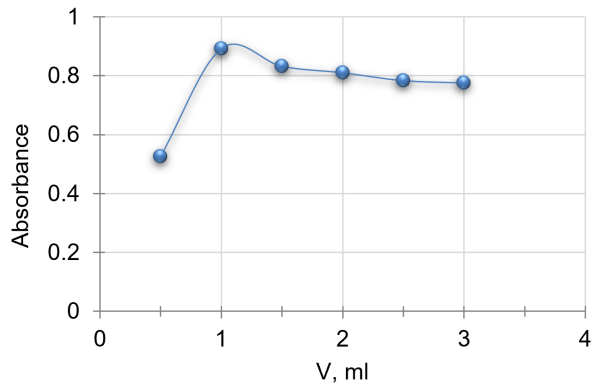

Fig. 2. Effect of potassium iodate (KIO_4) volume on the absorbance of the amiodarone–2,4-DNPH oxidative coupling product, C_{KIO_4} = 0.1 mol L^{-1}.

Effect of alkaline medium. The oxidative coupling reaction was significantly affected by the choice of alkaline medium. Sodium hydroxide, lithium hydroxide, and potassium hydroxide were each tested under identical conditions. Sodium hydroxide produced the highest absorbance and most consistent results. Potassium hydroxide caused turbidity, which interfered with spectral measurements (Fig. 3). Therefore, sodium hydroxide was chosen as the optimal alkaline medium for this method.

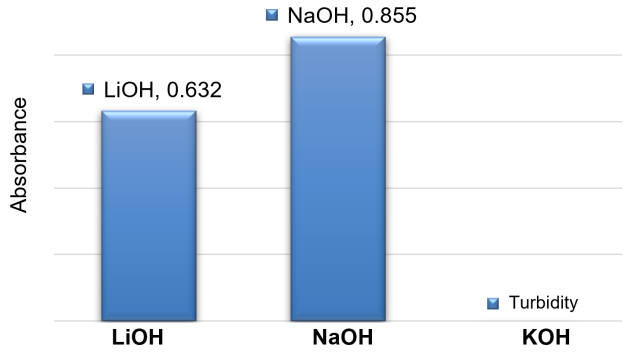

Fig. 3. Effect of alkaline medium on the absorbance of the amiodarone–2,4-DNPH oxidative coupling product, C_{base} = 0.1 mol L^{-1}.

Effect of reaction time. The effect of reaction time on color development and product stability was evaluated over the range of 5 to 50 minutes. Absorbance increased during the initial stage and reached a maximum at approximately 15 minutes. It then remained nearly constant, indicating stable chromogenic species (Fig. 4). Thus, a 15-minute reaction time was selected as optimal for routine analysis.

Effect of the order of reagent addition. The order in which the reagents were added strongly influenced the formation of the oxidative coupling product. As summarized in Table 1, the highest absorbance (0.852) was obtained when 2,4-dinitrophenylhydrazine was first mixed with potassium iodate, then with amiodarone, and finally with sodium hydroxide: 2,4-DNPH → KIO_4 → amiodarone → NaOH. This sequence was therefore adopted throughout the study to ensure maximum sensitivity and reproducibility.

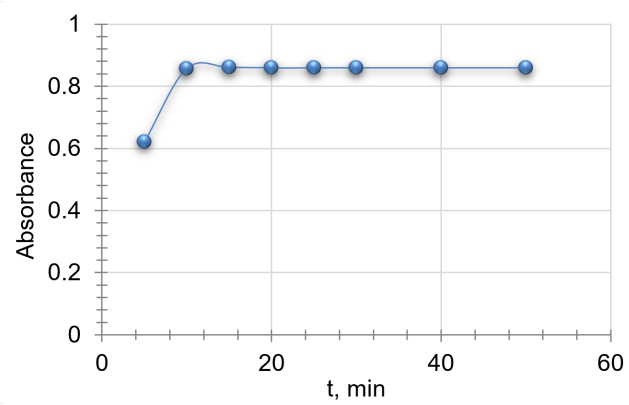

Fig. 4. Effect of reaction time on the absorbance of the amiodarone–2,4-DNPH oxidative coupling product.

Analytical performance of the proposed method. Under optimized experimental conditions, calibration solutions with different amiodarone concentrations were treated according to the recommended procedure, and absorbance was measured at 622 nm against the reagent blank. Key analytical characteristics of the proposed method, such as molar absorptivity, Sandell's sensitivity, detection limit, and quantification limit, are summarized in Table 2. The regression equation is y = 0.036x + 0.012. The correlation coefficient R^2 is 0.9932. Molar absorptivity and Sandell's sensitivity are 2.32 × 10^4 L mol^{-1} cm^{-1} and 0.027 μg cm^{-2}, respectively. The limits of detection (LOD) and quantification (LOQ) are 1.11 and 2.32 μg mL^{-1}, respectively. A linear relationship between absorbance and amiodarone concentration was obtained within the range of 1.5–30 μg mL^{-1}.

Table 1. Effect of the order of reagent addition on the absorbance of the reaction product.

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

Table 2. Analytical characteristics of the proposed spectrophotometric method for amiodarone determination.

Parameter	Value
λ_{max} (nm)	622
Color of product	yellowish green
Linearity range (μg mL^{-1})	1.5–30
Regression equation	y = 0.036x + 0.012
Correlation coefficient (R^2)	0.9932
Molar absorptivity (L mol^{-1} cm^{-1})	2.32 × 10^4
Sandell's sensitivity (μg cm^{-2})	0.027
Limit of detection (LOD, μg mL^{-1})	1.11
Limit of quantification (LOQ, μg mL^{-1})	2.32
Relative standard deviation (%)	1.75
Recovery (%)	99.87
Stoichiometric ratio	1:1

Precision and accuracy. The precision and accuracy of the developed analytical method were evaluated by measuring amiodarone at three concentration levels under identical experimental conditions. Precision was determined by relative standard deviation (RSD %), and accuracy by relative error (E %). The obtained RSD % values were lower than 2.1%, which indicates satisfactory repeatability and acceptable precision of the proposed procedure. Low relative error values confirm good accuracy for amiodarone quantification. Results are shown in Table 3.

Table 3. Precision and accuracy parameters for the determination of amiodarone by the proposed spectrophotometric method.

Taken (μg mL^{-1})	Found (μg mL^{-1})	E (%)	RSD (%)
5.0	4.90	−2.10	1.79
10.0	10.11	+1.13	2.05
20.0	20.36	+1.79	1.44

Application to pharmaceutical formulations. The proposed spectrophotometric method was successfully applied to the determination of amiodarone in commercial pharmaceutical tablet formulations. Tablet samples were analyzed according to the recommended analytical procedure under the optimized experimental conditions. As shown in Table 4, recoveries ranged from 100.53 to 102.00 %, demonstrating satisfactory accuracy and reliability of the method. The obtained results indicate that common tablet excipients did not significantly interfere with the determination of amiodarone under the selected experimental conditions.

Table 4. Determination of amiodarone in pharmaceutical tablet formulations.

Nominal concentration (μg mL^{-1})	Found concentration (μg mL^{-1})	Recovery (%)	RSD (%)
5.00	5.08	101.60	2.02
10.00	10.20	102.00	1.95
15.00	15.08	100.53	2.81

Green analytical chemistry assessment. The environmental sustainability of the proposed method was evaluated using the Analytical GREEnness (AGREE) metric, which is based on the 12 principles of Green Analytical Chemistry. Results are shown in Fig. 5. The AGREE score of 0.71 indicates good overall greenness and environmental compatibility of the developed analytical procedure. The favorable greenness profile can be attributed to several factors, including the use of water as the reaction medium, low reagent consumption, minimal energy requirements associated with UV–Vis spectrophotometric measurements, and the absence of organic extraction solvents. In addition, the method requires simple instrumentation, generates a relatively small volume of analytical waste, and can be performed within a short analysis time. Although the procedure uses an oxidative coupling reaction with 2,4-dinitrophenylhydrazine and potassium iodate, which slightly lowers its greenness score, its overall environmental impact is still much lower than that of many chromatographic methods that require large amounts of organic solvents and complex equipment. Therefore, the proposed method can be considered a practical, economical, and environmentally acceptable alternative for the routine determination of amiodarone in pharmaceutical formulations.

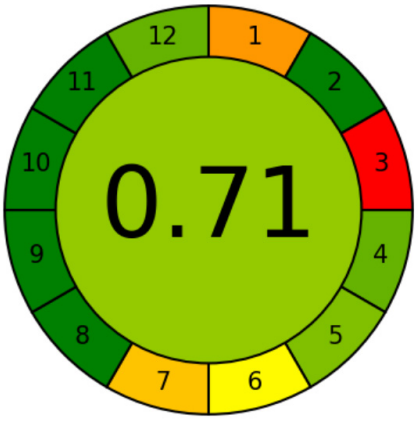

Fig. 5. AGREE assessment of the proposed spectrophotometric method for the determination of amiodarone.

Conclusion

A simple, sensitive, and cost-effective spectrophotometric method was developed to determine amiodarone via oxidative coupling with 2,4-dinitrophenylhydrazine in an alkaline medium. The method demonstrated good linearity, precision, and accuracy across the tested concentration range. It was successfully applied to pharmaceutical tablet analysis, yielding satisfactory recovery and reproducibility. Its simplicity, speed, and low cost make this procedure a practical alternative for routine quality control of amiodarone in pharmaceutical preparations.

Authors' contributions

Isam Mohammed Turki and Fatima Hamza M. Baker wrote the manuscript. Mohammed K. Kahlol checked and validated the results. Muthana S. Mashkoor reviewed the manuscript. All authors read and approved the final version.

Data availability

Data will be made available on request.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

Declaration of AI-assisted technologies

During the preparation of this work, the authors used ChatGPT 5.0, a language model developed by OpenAI, to support text drafting and editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

References

1. Șorodoc, V.; Indrei, L.; Dobroghi, C.; Asaftei, A.; Ceasovschih, A.; Constantin, M.; Lionte, C.; Morărașu, B. C.; Diaconu, A.-D.; & Șorodoc, L. Amiodarone Therapy: Updated Practical Insights. *J. Clin. Med.* 2024, 13(20), 6094. https://doi.org/10.3390/jcm13206094

2. Connolly, S. J. Evidence-Based Analysis of Amiodarone Efficacy and Safety. *Circulation.* 1999, 100(19), 2025–2034. https://doi.org/10.1161/01.CIR.100.19.2025

3. Hamilton, D.; Nandkeolyar, S.; Lan, H.; Desai, P.; Evans, J.; Hauschild, C.; Choksi, D.; Abudayyeh, I.; Contractor, T.; & Hilliard, A. Amiodarone: A Comprehensive Guide for Clinicians. *Am. J. Cardiovasc. Drugs.* 2020, 20, 549–558.

4. Rodríguez-Fernández, K.; Gras-Colomer, E.; Climente-Martí, M.; Mangas-Sanjuán, V.; & Merino-Sanjuán, M. Pharmacometric Characterization of Entero-Hepatic Circulation Processes of Orally Administered Formulations of Amiodarone under Complex Binding Kinetics. *Eur. J. Pharm. Sci.* 2022, 174, 106198. https://doi.org/10.1016/j.ejps.2022.106198

5. Colunga Biancatelli, R. M. L.; Congedo, V.; Calvosa, L.; Ciacciarelli, M.; Polidoro, A.; & Iuliano, L. Adverse Reactions of Amiodarone. *J. Geriatr. Cardiol.* 2019, 16(7), 552–566. https://doi.org/10.11909/j.issn.1671-5411.2019.07.004

6. Al-Hiari, M.; Abumuhfouz, M.; Kayali, L.; Panta, U.; & Rueda Rios, C. A Fatal Consequence: Amiodarone-Induced Multiorgan Toxicity. *Cureus.* 2024, 16(6), e62260. https://doi.org/10.7759/cureus

7. Lv, H.-J.; & Zhao, H.-W. Amiodarone-Induced Hepatotoxicity—Quantitative Measurement of Iodine Density in the Liver Using Dual-Energy Computed Tomography: Three Case Reports. *World J. Clin. Cases.* 2020, 8(20), 4958–4965. https://doi.org/10.12998/wjcc.v8.i20.4958.

8. Siddoway, L. A. Amiodarone: Guidelines for Use and Monitoring. *Am. Fam. Physician.* 2003, 68(11), 2189–2196.

9. Hoofnagle, J. H. Amiodarone. In LiverTox®: Clinical and Research Information on Drug-Induced Liver Injury; National Institute of Diabetes and Digestive and Kidney Diseases: Bethesda, MD, 2026. https://www.ncbi.nlm.nih.gov/books/NBK548109/

10. Florek, J. B.; Lucas, A.; & Girzadas, D. Amiodarone. *StatPearls* [Internet]. StatPearls Publishing: Treasure Island (FL), 2023. https://www.ncbi.nlm.nih.gov/books/NBK482154/

11. Juenke, J. M.; Brown, P. I.; McMillin, G. A.; & Urry, F. M. A Rapid Procedure for the Monitoring of Amiodarone and N-Desethylamiodarone by HPLC-UV Detection. *J. Anal. Toxicol.* 2004, 28(1), 63–66. https://doi.org/10.1093/jat/28.1.63

12. Pérez-Ruiz, T.; Martínez-Lozano, C.; & García-Martínez, M. D. Simultaneous Determination of Amiodarone and Its Metabolite Desethylamiodarone by High-Performance Liquid Chromatography with Chemiluminescent Detection. *Anal. Chim. Acta.* 2008, 623(1), 89–95. https://doi.org/10.1016/j.aca.2008.06.003

13. Kollroser, M.; & Schober, C. Determination of Amiodarone and Desethylamiodarone in Human Plasma by High-Performance Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry with an Ion Trap Detector. *J. Chromatogr. B.* 2002, 766(2), 219–226. https://doi.org/10.1016/S0378-4347(01)00469-8

14. Rahman, N.; Haque, S. K. M.; & Azmi, S. N. H.; Rahman, H. Optimized and Validated Spectrophotometric Methods for the Determination of Amiodarone Hydrochloride in Commercial Dosage Forms Using N-Bromosuccinimide and Bromothymol Blue. *J. Saudi Chem. Soc.* 2017, 21(1), 25–34. https://doi.org/10.1016/j.jscs.2013.09.001

15. Stefan, R.-I.; Aboul-Enein, H. Y.; & Baiulescu, G.-E. Amiodarone-Selective Membrane Electrode. *Sens. Actuators, B.* 1996, 37(3), 141–144. https://doi.org/10.1016/S0925-4005(97)80129-3

16. Banan, K.; Niknam, S.; Ahmadi, M.; Tabasi, S.; Ghalkhani, M.; Adabi, M.; & Ghorbani-Bidko-

reph, F. Molecularly Imprinted Electrochemical Sensor Based on Carbon Nanofibers for Amiodarone Determination. *Microchem. J.* 2024, 200, 110365. https://doi.org/10.1016/j.microc.2024.110365

17. Doğan, S.; & Levent, A. Investigation of Electrochemical Properties and Detection of the Antiarrhythmic Drug Amiodarone in Pharmaceutical and Urine Samples Using a Disposable Pencil Graphite Electrode. *Ionics.* 2025, 31(6), 6353–6364. https://doi.org/10.1007/s11581-025-06298-x

18. El Sheikh, R.; Gouda, A. A.; Ghazy, A. A.; Jumaa, N. M.; Elsaify, N. E. M.; Soliman, N. S. A.; El Sayed, A.; Moustafa, A. H.; Babalghith, A. O.; El Sayed, A.; & Abd El-Majeed, A.-S. M. Development and Validation of Spectrophotometric Methods for Estimation of Antiarrhythmic Drug Dronedarone Hydrochloride in Pure and Dosage Forms. *Talanta Open.* 2024, 10, 100355. https://doi.org/10.1016/j.talo.2024.100355