NTU Journal of Pure Sciences
Double Blind Peer Review Journal
ISSN: 2789-1089 EISSN: 2789-1097
Available Online: https://journals.ntu.edu.iq/index.php/NTU-JPS
Volume 5, No. 1, 2026

Original Article

Eco-friendly Indirect Spectrophotometric Method for Estimation of Amiloride Hydrochloride in its Pharmaceutical Preparations Via Eosin Y

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Received: 14 August 2025
Accepted: 06 February 2026
Published: 12 April 2026
DOI: https://doi.org/10.56286/t1f5hk02
Article ID: 1596

Abstract

A simple, sensitive, and precise indirect spectrophotometric method has been developed for quantifying amiloride hydrochloride (AMD. HCl), both as a pure compound and in its dosage forms (tablets). This work is based on indirect spectrophotometric method of the oxidation of (AMD. HCl) by adding a known amount of oxidizing agent calcium hypochlorite ($Ca(OCl)_2$) in acidic medium. The unreacted amount of $Ca(OCl)_2$ then reacted with a known quantity of the dye eosin Y to shorten its color, and then the absorption of the remaining amount of the dye to form an orange-colored product that can be measured at a wavelength of 518 nm. A thorough investigation was conducted into the optimization of method conditions. Beer's law was followed under ideal circumstances in the concentration range of 2–12 µg/mL, with a relative standard deviation of 2.62%, a recovery of 100.03%, and a determination coefficient (R^2) of 0.9992. The limit of quantification (LOQ) was 0.0932 µg/mL, while the limit of detection (LOD) was 0.028 µg/mL. The molar absorptivity coefficient was 1.8856×10^4 L/mol. cm, which corresponds to Sandall's sensitivity of 0.0138 µg/cm^2. The method was applied to the assay amiloride hydrochloride (AMD. HCl) in tablets, yielding acceptable analytical results. Additionally, a new tool for assessing analytical protocols related to green analytical chemistry attributes had been used in order to assess the environmental impact of the proposed technique. The new instrument is known as the Green Analytical Procedure Index, or GAPI.

Keywords: Amiloride hydrochloride, Eosin Y dye, Calcium hypochlorite, Indirect Spectrophotometry, GAPI

Introduction

Amiloride hydrochloride (AMD. HCl) is a potassium-sparing diuretic. It is chemically 3,5-diamino-N-(diaminomethylene)-6-chloropyrazine-carboxamide-monohydrochloride-hydrate [1,2]. In addition, it inhibits sodium reabsorption across the epithelial sodium channels in the distal convoluted tubules and collecting channels of the kidney. At present, amiloride is specified in oedematous states, and for potassium conservation, adjunctive to thiazide or loop diuretics for hypertension, hepatic cirrhosis with ascites, and congestive heart failure [3-5]. Figure 1. Explains the chemical structure of AMD. HCl [6]

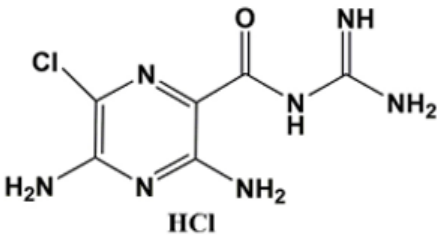


Figure 1. Structure of amiloride hydrochloride

Amiloride can only be taken orally as tablets, and it takes two hours for it to start working and three to four hours for its plasma levels to reach their peak [7]. In the literature, there are many methods to estimate AMD. HCl, including the following: RP-HPLC method [8-11], RP-UPLC method [12], spectrophotometry [13-15], spectrofluorometric analysis [16,17], and voltammetry [18,19]. A few spectrophotometric methods for the determination of amiloride hydrochloride have been reported in the literature. This technique, which is known as eosin Y because of its yellowish-red hue, is based on the oxidation of the eosin Y dye in the presence of acid in the aqueous medium, Xanthene dye 2-(2,4,5,7-tetrabromo-3-oxido-3H-xanthen-9-yl) benzoate, primarily as a coloring reagent in light-sensitive photopolymerization processes, the food industry, pharmaceuticals, cosmetics, biological analysis, and textiles. Its molecular formula is $C_{20}H_6Br_4Na_2O_5$, and its molecular weight is 691.85 g/mol[20-22]. It is referred to as an acidic dye because it only contains one carboxyl group. In an acidic medium, it can form ion-pair complexes with basic medications [23]. Figure 2. explains the chemical structure of eosin Y [24].

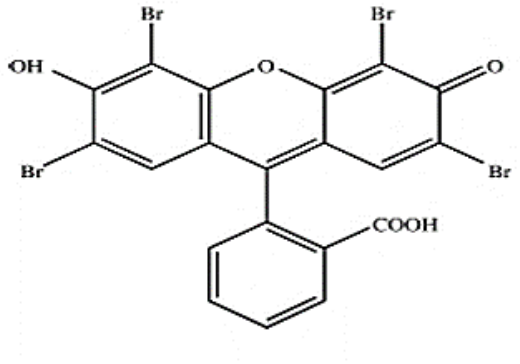


Figure 2. The chemical structure of eosin Y

The oxidizing agent used in this method was calcium hypochlorite Ca(OCl)2, which is a granulated powder with a larger amount of available chlorine and more stability [25]. As benefits, Ca(OCl)2 appears to result in fewer structural alterations in dentin, has no negative effects of adhesive procedures, and does not produce harmful byproducts when it reacts with other endodontic solutions [26]. Recently, because calcium hypochlorite solution (Ca(OCl)2) has antimicrobial properties and dissolves organic tissue, it has been suggested as an endodontic irrigant[27]. This study presents a highly sensitive indirect spectrophotometric method for the determination of AMD. HCl in an acidic medium. This study aimed to develop a straightforward, quick, accurate, and precise indirect spectrophotometric method for the estimation of AMD. HCl in pure and tablet dosage form with eosin Y, and to validate the environmental impact of the suggested technique.

Experimental

Instruments

All spectral measurements and absorption readings were conducted using a JASCO-360 spectrophotometer. Featured with 1cm glass, cells were utilized for the experiments. The pH was measured by a TRANS BP3001 pH meter, and ABS-120-4 by a Kern and Sohn sensitive balance was used for the necessary weighing operations.

Chemical reagents and standard solution

Every chemical used was of the caliber of an analytical reagent, and the pure amiloride hydrochloride was provided by the SDI company (a provider for drug industries and medical appliances, Samarra, Iraq).

Amiloride hydrochloride (100 µg/mL):

This was made by completely dissolving 0.01 g in warm distilled water using a stirrer. Then, it was diluted to 100 mL in a volumetric flask and kept protected from sunlight in an ambient bottle [28].

Eosin Y (100 µg/mL) (Merck):

It was prepared by dissolving 0.01 g in distilled water and filling the volume up to the mark in a 100 mL volumetric flask.

Calcium hypochlorite (1×10^{-3} M):

It was prepared by weighing 0.01430 g and dissolved in distilled water with heating in a water bath and continuous stirring until dissolved completely, and then the volume was completed to the mark in a 100 mL volumetric flask.

Acetic acid (0.2 M) (BDH):

1M of standard solution, acetic acid diluted 5.75 mL of conc. acid (17.4 M) with distilled water to a final volume of 100 mL in a volumetric flask was prepared. A working solution of 0.2 M was produced by appropriately diluting the stock solution.

Tablets analysis of amiloride hydrochloride:

The solutions were prepared by weighing five Awaretic Tablets (Irbil, Iraq) and five Saluretic tablets (Kahira, Egypt), each containing 5 mg of AMD. HCl. The Tablets were ground and mixed to obtain 0.0100 grams of pure AMD. HCl, then dissolved in distilled water using heat and stirring. The solution volume was adjusted to 100 mL in a volumetric flask and filtered to achieve a concentration of 100 µg/mL of AMD. HCl, stored in a dark bottle to protect it from light.

Results & Discussion

Principle of method

The principle of the method was based on the oxidation of AMD. HCl by adding a known amount of oxidizing agent, calcium hypochlorite ($Ca(OCl)_2$) in an acidic medium, leaving an unreacted amount of the agent as shown in equation 1:

$$\underset{\text{(known)}}{\text{Amiloride}} + \underset{\text{(excess)}}{Ca(OCl)_2} \xrightarrow{H^+} \underset{\text{(oxidized form)}}{\text{Amiloride}} + \underset{\text{(unreacted)}}{Ca(OCl)_2} \quad (1)$$

The subsequent determination of the unreacted oxidant agent was done by decolorization of eosin Y dye and measuring the absorbance of unoxidized dye at 518 nm asequation 2 shows:

$$\underset{\text{(unreacted)}}{Ca(OCl)_2} + \underset{\text{(excess)}}{\text{Eosin Y}} \xrightarrow{H^+} \underset{\text{(oxidized dye)}}{\text{Eosin Y}} + \underset{\text{(unreacted)}}{\text{Eosin Y}}$$

$$\text{(Absorbed at 518 nm)} \quad (2)$$

Absorption spectrum and calibration graph of the dye

The absorption spectrum of (100 µg/mL) eosin Y was studied in the presence of 0.2 M acetic acid, which gave the best absorption at a wavelength of 518 nm. As shown in Figure 3.

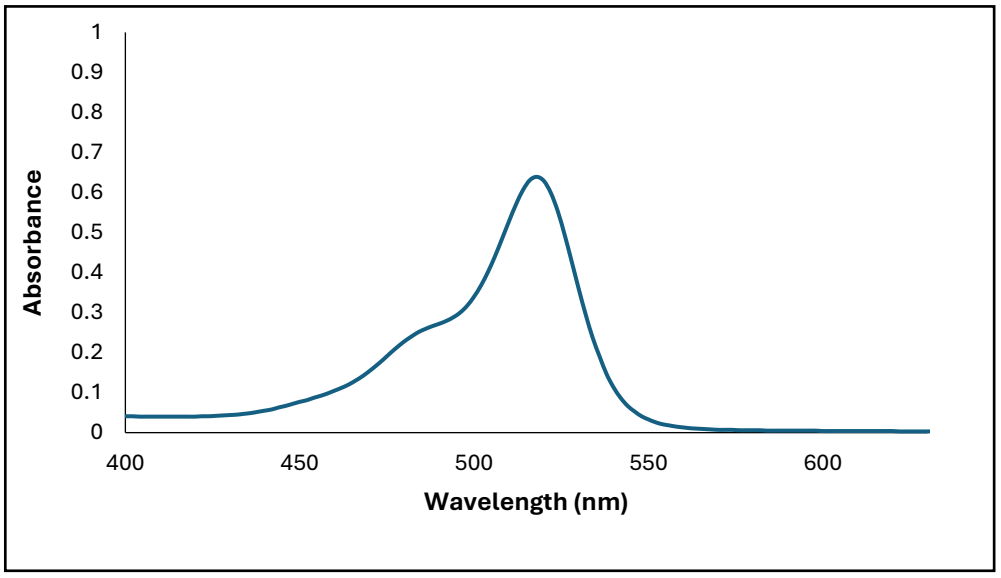


Figure 3. Absorption spectrum of Eosin Y dye

Additionally, the Eosin Y calibration graph was studied using varying volumes (0.1–1.5 mL) of dye (100 µg/mL) in 10 mL volumetric flasks. One milliliter of acetic acid (0.2 M) was then added, and the volume was completed with distilled water. The absorption intensity of the dye was measured at 518 nm.Also, 1 mL of eosin Y was selected for the following experiments and Figure 4. indicates the calibration graph of the dye.

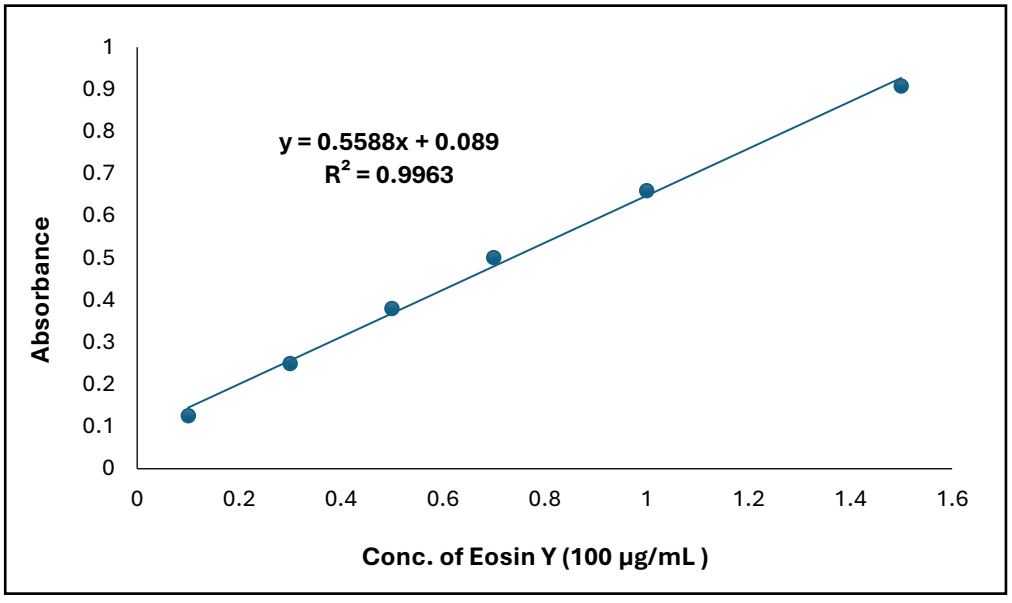


Figure 4. Calibration graph of Eosin Y dye

Setting the optimum conditions

Choosing the type of oxidizing agent

To obtain the best decolorization of eosin Y dye, a set of oxidizing agents (1×10^{-3} M) is shown in Figure 5. The absorption spectrum was measured for each of them at 518 nm; calcium hypochlorite (1×10^{-3} M) provided the best decolorization of the dye, which was selected in the next experiments.

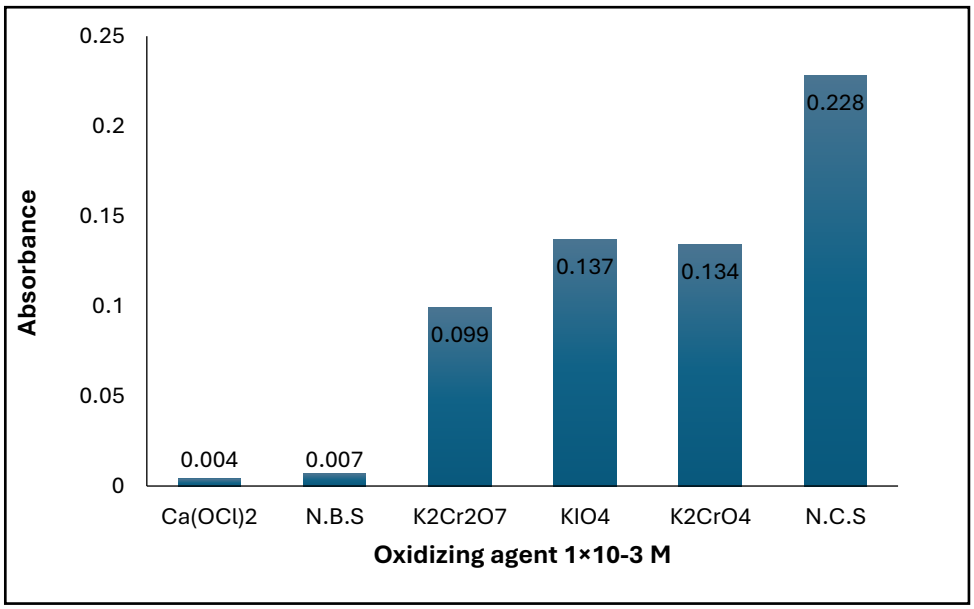


Figure 5. Effect of various oxidizing agents

The effect of 1×10^{-3} M calcium hypochlorite amount on absorbance

Different volumes (0.1-2.0 mL) were added to the reaction medium, in a final volume of 10 mL. Then, the absorption of each sample was measured at 518 nm; the best volume of calcium hypochlorite was 1.5 mL, as shown in Figure 6.

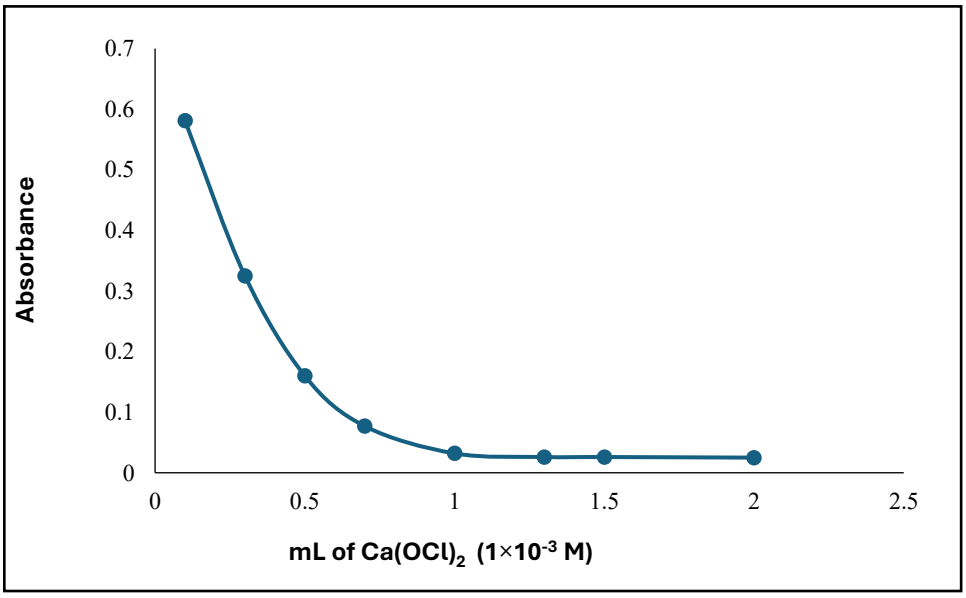


Figure 6. Effect of the different amounts of oxidizing agent

Different types of acid

1 mL of different types of (0.2 M) acids was added to 1 mL of (100 µg/mL) drug with 1.5 mL of (1×10^{-3} M) oxidizing agent $Ca(OCl)_2$ and wait for 5 min, then 1 mL of (50 µg/mL) dye was added and wait for 5 min, filling the volume with distilled water to the mark in

a 10 mL volumetric flask. Table 1. shows that the best acid was CH_3COOH, which gave the highest absorbance value, and it was selected in the next experiments.

Table 1. Effect of the different types of acids

Acid	*Abs*	*λ max*	*PH*	*Abs of blank*
CH_3COOH	0.277	518 nm	2.81	0.001
HCl	0.068	484 nm	1.58	-0.001
H_2SO_4	0.064	484 nm	1.68	-0.000
HNO_3	0.066	484 nm	1.62	0.000
H_3PO_4	0.073	484 nm	2.11	0.001

Effect of the amount of 0.2 M acetic acid on absorbance

Various volumes (0.2-1.0 mL) of CH_3COOH were added to several volumetric flasks containing different concentrations (4-12 µg/mL) of pure amiloride hydrochloride solution. The remaining components of the reaction were added according to the previously stated method with the specified volumes and concentrations. The results are shown in Table 2. 0.3 mL of 0.2 M CH_3COOH was the optimal volume that gave the highest value for the coefficient of determination ($R^2 = 0.9941$), which was selected in the next experiments.

Table 2. Effects of various acetic acid volumes

mL of (0.2M) CH_3COOH	*Absorbance / µg of AMD. HCl in 10 mL*					*R^2*
	4	**6**	**8**	**10**	**12**	
0.2	0.039	0.247	0.525	0.752	0.830	0.9738
0.3	0.056	0.201	0.375	0.524	0.630	0.9941
0.4	0.073	0.252	0.415	0.535	0.608	0.9747
0.5	0.052	0.222	0.373	0.509	0.591	0.9854
0.7	0.057	0.224	0.410	0.525	0.575	0.9598
1.0	0.077	0.220	0.360	0.491	0.533	0.9722

Oxidation time

The oxidation time was studied by added 1.5 mL of (1×10^{-3} M) $Ca(OCl)_2$ to 1 mL of (100µg/mL) (AMD. HCl), then added 0.3 mL of (0.2 M) CH_3COOH, the solution was left for different period, before adding 1 mL of (100µg/mL) eosin Y. After that, it was diluted with distilled water to the mark and measured the absorbance at 518 nm against blank solution. The result was shown in Table 3.

Table 3. Effect of Oxidation Time

Standing time before addition (100µg/mL) eosin Y	*Absorbance/ Standing time after adding eosin Y and before dilution, min.*			
	5	10	15	20
5	0.622	0.532	0.540	0.466
10	**0.639**	0.621	0.565	0.562
15	0.631	0.549	0.546	0.515
20	0.613	0.517	0.497	0.512

The effect of temperature and stability time

Different temperatures between 0 and 40°C were studied for the formation and stability of the product. Room temperature (23±2°C) was the optimal temperature for estimation with a stabilization time of at least one hour; the results are shown in Figure 7 in the subsequent experiments.

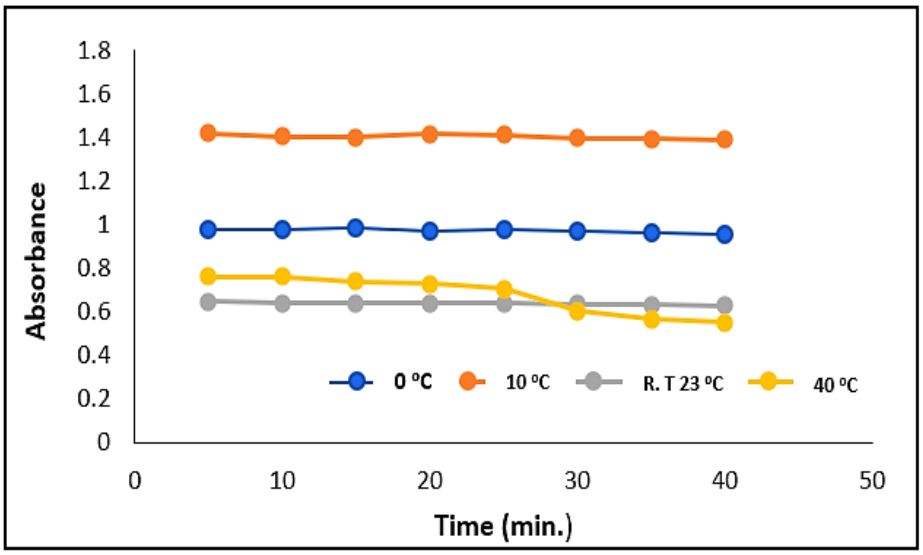


Figure 7. Effect of temperature and stability time

Effect of addition sequence

The various addition sequences were examined. Table 4 results show that the first order was optimal since it produced the highest absorbance value; as a result, it is advised for use in the experiments that follow.

Table 4. Effect of addition sequence

Number of Order	*Reaction component*	*Absorbance*
I	*S+OX+A.A+EY	0.640
II	*S+A.A+OX+EY	0.322
III	*S+OX+EY+A.A	0.586
IIII	*S+A.A+EY+OX	0.237

*(S) Drug, (OX) $Ca(OCl)_2$, (A.A) CH_3COOH, (EY) Eosin Y Dye

Effect of the surfactant on absorbance

To study the influence of surfactants on the intensity of dye absorption, 1 mL of different surfactants was added to a reaction medium with a final volume of 10 mL. The surfactants included negatively charged (SDS), positively charged (CTAB and CPC), and neutral surfactants (Triton X-100). They were disregarded because there was no effect on absorbance intensity.

Final absorption spectrum

The reactions were then altered to perfect conditions, as shown in Figure 8. The absorption spectrum was obtained.

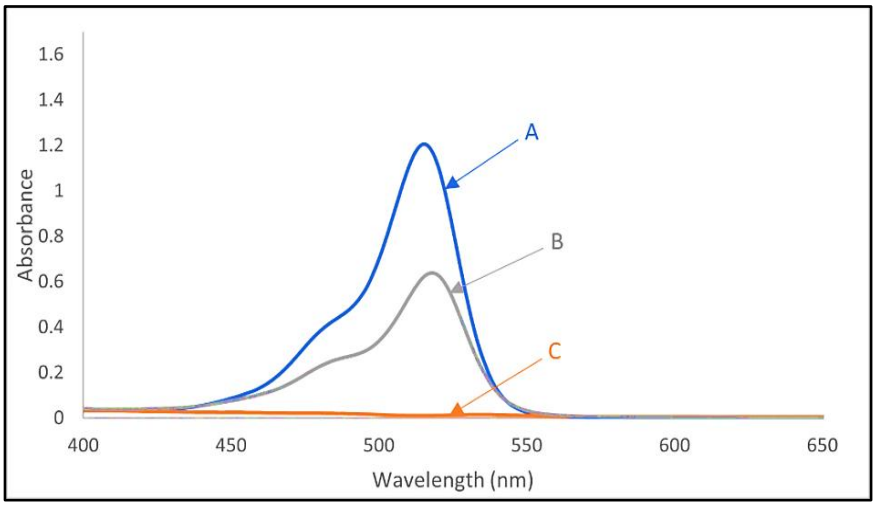


Figure 8. Final spectrum, (A) Eosin Y dye, (B) Eosin Y residue after oxidizing AMD. HCl vs blank solution, (C) blank solution vs D.W

Calibration graph

Plotting absorbance against AMD HCl concentration produced a linear calibration graph under ideal conditions. Within the concentration range of 2 to 12 µg/mL of AMD. HCl in a final volume of 10 mL, this graph is consistent with Beer's law. The graph's estimated coefficient is R^2 = 0.9992. According to Sandall's sensitivity of 0.0138 µg/cm^2, the estimated molar absorptivity was 1.8856×104 L/mol·cm.. As shown in Figure 9.

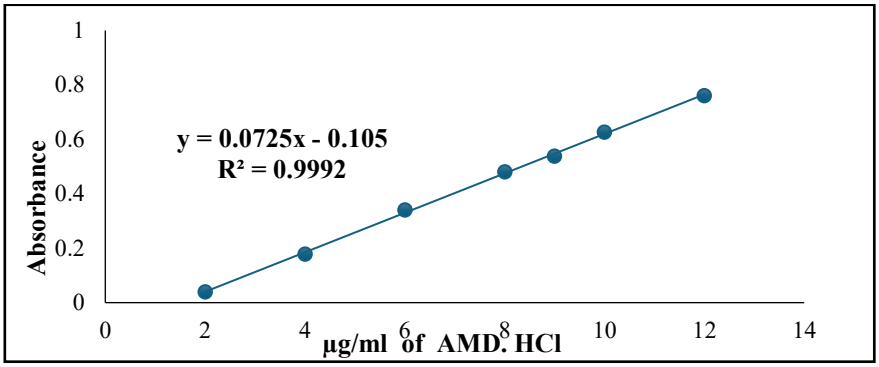


Figure 9. Calibration graph for the determination of AMD. HCl

Interference studies

Through the analysis of a synthetic solution containing 10 µg/mL of AMD, the effects of common excipients in the pharmaceutical preparations were investigated, when three amounts are present (25, 50, and 100 µg/mL) of glucose, sucrose, and Arabic gum in the final volume of 10 mL. There were no interferences observed in the estimation of AMD. HCl in the presence of the common excipient.

The optimal conditions

The optimal conditions are recorded in Table 5.

Table 5. An overview of optimal conditions

Parameters	*Optimum conditions*
Dye used	Eosin Y
Amount of (100 µg/mL), Eosin Y, mL	1
Oxidizing agent	$Ca(OCl)_2$
Amount of (1×10^{-3} M), $Ca(OCl)_2$, mL	1.5
Type of Acid	CH_3COOH
Amount of (0.2 M), Acid, Ml	0.3
Linearity (µg/mL)	2-12
molar absorptivity (L/mol·cm)	1.8856×10^4
Sandall's sensitivity (µg/cm^2)	0.0138
Temperature (°C)	23±2
Solvent	Water
λ max (nm)	518
Oxidation time, (min)	10
Bleaching time, (min)	5
Medium	aqueous

Precision and Accuracy of the Method

Two different concentrations of AMD. HCl were determined for the proposed method. The results are shown in Table 6. Good precision and accuracy were achieved with the suggestion of this method.

Table 6. Accuracy and precision of the suggested method

Drug	*Amount taken (µg/10 mL)*	*Recovery (%)**	*Average Recovery (%)**	*RE %**	*RSD %**
Amiloride hydrochloride	6	99.16	100.03	-0.84	3.50
	10	100.90		0.9	1.74

*Average of five determinations

Application

This method was applied for the standard solution of 100 µg/mL of AMD. HCl in its pharmaceutical preparations (Tablets) for two different concentrations, 6 and 10 µg/mL. Analytical application results are in Table 7, and they were preserved according to the method described work for the standard solutions.

Table 7. Application of the method to pharmaceutical preparations

Drug	*Amount takes (µg/ mL)*	*Amount found (µg/ mL)*	*Recovery (%)**	*RE %**	*RSD %**	*t-exp (N=3)*
Awaretic 5mg tablet Erbil-Iraqi	6	6.12	102.0	2.0	1.80	1.210
	10	10.11	101.1	1.1	0.70	1.026
Saluretic 5mg tablet Kahira-Egypt	6	6.21	103.5	3.5	1.47	0.246
	10	10.02	100.2	0.2	0.65	0.742

*Average of five determinations t-exp[a]: t-experimental, $t - \exp \pm = \frac{\sqrt{N}}{s} \ (\bar{X} - M)$

From the results recorded in Table 8, it can be concluded that the rate of recovery for AMD. HCl Tablets analysis was 101.55% for Awaretic and 101.85% for Saluretic, which shows that the method has good accuracy and efficiency in determination. The t-test value [29] was calculated for the concentrations of 6 and 10 µg/mL for tablets of Awaretic and Saluretic companies; the t-test value is less than the tabular value with degrees of freedom three (N=3) and at a 95% confidence level, which indicates the success of the method.

Standard addition

To demonstrate the established method and its achievement in estimation and the additive interferences of its free, the standard addition method was applied to the preparation of pharmaceuticals, as shown in Figure 10.

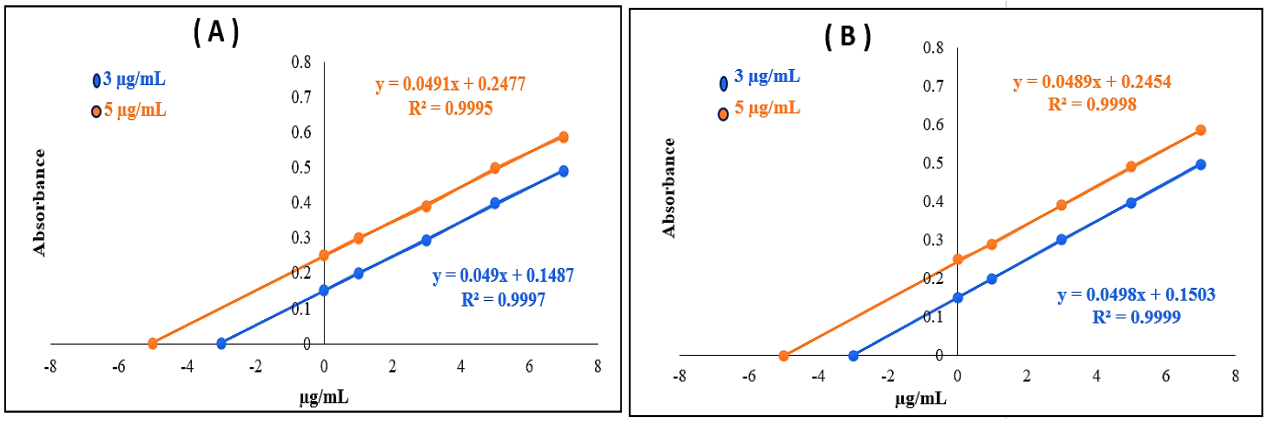


Figure 10. The standard addition of the method, (A) Awaretic 5mg tablet Erbil-Iraqi, (B) Saluretic 5m tablet Kahira-Egypt

Table 8. The standard addition of the method results for amiloride hydrochloride (AMD. HCl)

Drug	*Amount taken (µg/ ml)*	*Amount found (µg/ ml)*	*Recovery (%)*	*RE %*
Awaretic 5mg tablet Erbil-Iraqi	3	2.92	97.33	-2.67

Saluretic 5mg tablet Kahira-Egypt	5	4.99	99.8	-0.2
	3	3.03	101.0	+1.0
	5	4.92	98.4	-1.60

Comparison with other methods

A number of analytical parameters from the recent spectrophotometric method and the current method are compared in Table 9.

Table 9. Comparison with the other methods.

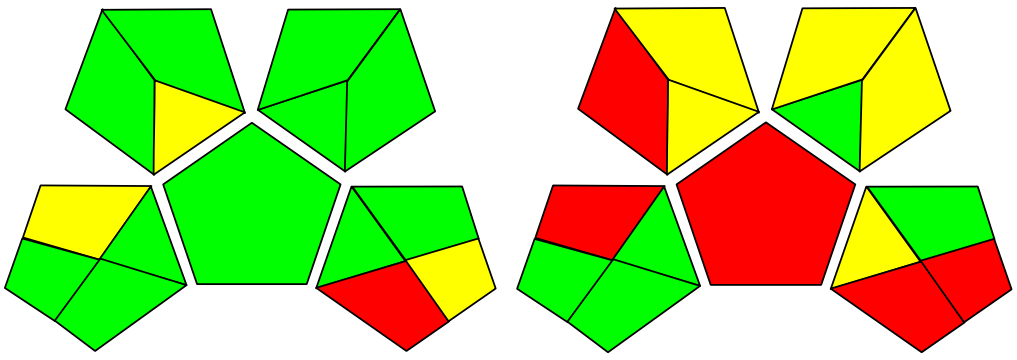
Analytical parameters	*Present Method*	*Literature Method [30]*	*Literature Method [30]*
Reagent	Eosin Y dye	P-chloranilic acid	DDQ
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1.9478×10^4	0.204×10^4	0.331×10^4
Sandall's sensitivity (µg/cm2)	0.0137 µg/cm^2	11.2500 ng/cm^2	6.9592 ng/cm^2
Beer's law Range (µg/mL)	2-12	1-6	5-30
Temperature (^{0}C)	At room Temperature 23±2	28	28
λmax (nm)	518	508	467
RSD (%)	2.62	0.118	0.940
L.O.D	0.0280	0.0140	0.7660
L.O.Q	0.0932	0.0470	2.553

Greenness assessment

Analytical chemists face the challenge of developing environmentally safe, user-friendly, cost-effective, and highly efficient techniques for the analysis of red tape. Green analytical chemistry's overarching goal is to encourage eco-friendly practices and minimize the use of hazardous materials [31]. Consequently, the two analytical tools used in the suggested procedures were thoroughly evaluated for their greenness. [32, 33].

(Green Analytic Procedure Index) GAPI method

The Green Analytical Procedure Index (GAPI), a novel tool that assesses the overall greenness of the analytical process technique using 15 distinct parameters, is depicted in Figure 11. Five pentagrams, each divided into several sections, were produced based on the level of ecological influence. Red and yellow denote high and medium environmental effects, respectively, while green shows moderate environmental effectiveness [33, 34].



a) b)

Figure 11. Pictograms from (a) the suggested method approaches and (b) other HPLC [35] method publications are compared analytically

Eco-scale analytical technique

The analytical eco-scale is one of the greenness assessment tools that is effective at evaluating the technique's performance and gathering quantifiable information about its environmental suitability while accounting for the tools used, waste produced, and chemical use. This tool is necessary to calculate the analytical eco-scale value, which is determined by adding all of these points to all environmental-harming elements and deducting from 100. An excellent green profile is on the horizon when the eco-scale score is 85 or higher. Capable green analysis is indicated by an aggregate score above 50, whereas insufficient green analysis by a score below 50 [36]. Table 10 reveals the eco-scale importance results based on a comparison between the suggested methodology and the HPLC method found in the literature.

Table 10. The proposed approaches' analytical eco-scale penalty points are contrasted with those of the approaches found in the literature.

Reagents	*Penalty points of the Proposed methods*	*Penalty points of the Literature HPLC method [35]*
Technique	0	0
Acetic acid	2	……..
Acetic acid (glacial)	……	4
Phosphoric acid	…….	……..
Sodium hydroxide	2	……
Acetonitrile	……	8
Eosin Y	0	…….
Instrument		
Energy	0	2
Oven	……..	……..
Wastes	3	5
Hazard	1	3
Net penalty marks	8	22
Total score (Analytical eco-scale)	92	78

Conclusion

The determination of amiloride hydrochloride (AMD. HCl) was developed by using an indirect spectrophotometric method, which was both accurate and sensitive; it depended on the eosin Y dye in an aqueous medium with an acid present. The colored formation's maximum absorption occurred at 518 nm. The technique was successfully used to prepare the medication (tablets). Finally, using the green analytical procedure index and analytical eco-scale approaches as a measure of the "greenness" profile, an evaluation of the suggested method's environmental impact was carried out. For routine and quality control analysis of amiloride hydrochloride, this approach is advised.

Acknowledgments

For providing the required tools and facilities, the authors would like to thank the Chemistry Department of the College of Science at the University of Mosul in Mosul, Iraq.

Conflicts Of Interest

Regarding the publication of this article, the authors declare that they have no conflicts of interest.

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