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Utilization of Localized Surface Plasmon Resonance of Silver Nanoparticles for the Spectrophotometric Estimation of Amlodipine and Hydrochlorothiazide

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Abstract

A potent long-acting calcium channel blockers is amlodipine. Patient with high blood pressure can take it to treat high blood pressure, angina pectoris, and reduce the risk of stroke. Hydrochlorothiazide, a diuretic, is used to treat edema and hypertension. Additionally, it is used to treat certain types of diabetes, hyperglycemia and hypokalemia. These drugs are widely available in the market and are commonly used orally. Therefore, a rapid, accurate, and inexpensive method for the determination of amlodipine and hydrochlorothiazide was developed and validated. This method is based on the ability of these drugs to reduce Ag^{+1} to Ag^{0} by forming silver nanoparticles (AgNPs) in the presence of sodium dodecyl sulfate as a stabilizing agent .Significant surface plasmon resonance of synthesized nanoparticles was observed at 418 and 420 nm wavelengths which was used for quantitative spectrophotometric determination of amlodipine and hydrochlorothiazide .The linear concentration ranges for amlodipine and hydrochlorothiazide were 0.5-28 and 0.8-6 µg mL^{-1}, with detection limits of 0.442 and 0.128 µg mL^{-1}. The proposed method successfully determined amlodipine and hydrochlorothiazide in pure and commercial formulation.

Keywords: amlodipine; hydrochlorothiazide; silver nanoparticles; surface plasmon resonance; spectrophotometry.

Introduction

The scientific name of amlodipine (AM) is (±)-2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl)-3-ethoxy carbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine. It has the following chemical structure Fig. 1, the molecular formula is $C_{20}H_{25}ClN_2O_5$, and the molar mass is 408.879 g/mol. It is a white powder slightly soluble in water and isopropanol, moderately soluble in alcohol, and freely in methanol[1].

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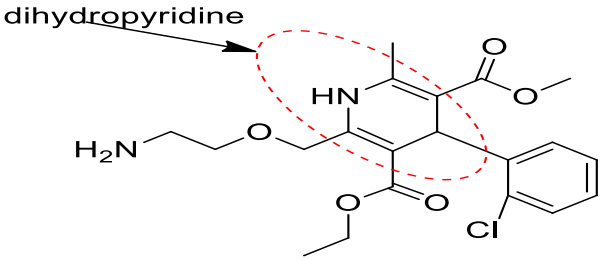

Figure 1. Structure of Amlodipine

AM is official in United States Pharmacopoeia, Indian Pharmacopoeia, and British Pharmacopoeia[2-3]. AM is the most prevalent class of dihydropyridine calcium channel blockers; it is a derivative of 1,4-dihydropyridine, which acts as an L-type calcium channel in the peripheral arterioles and lowers blood pressure by lowering total peripheral resistance. AM treats high blood pressure and chronic stable angina (chest pain or discomfort, usually associated with activity or stress, due to poor blood flow through the coronary arteries to the heart muscle)[4]. According to the literature review, many spectrophotometric methods have been published to evaluate AM in pure form, dosage forms, and biological fluids, including oxidation with potassium permanganate in an acidic environment[5], oxidation with Fe (III), and ammonium heptamolybdate tetrahydrate[6]. The charge transfer complexion reaction between AM and 2,3-dichloro5,6-diciano1,4-benzoquinone, p-chloroauric acid, and tetrachloquinone were developed [7,8]. The UV-Spectrophotometery method was proposed for the estimation of AM in a tablet [9]. Thiourea-based ligand (TU_3), N-((4′-methoxy-2′-nitrophenyl) carbamothioyl) dodecanamide, conjugated with AgNPs (TU_3-AgNPs) was explored as a spectrophotometric sensor that found to be selective for an antimicrobial drug AM[10]. A portable reflective absorbance spectrophotometric smartphone device determined AM by charge-transfer complex with picric acid[11]. An electroanalytical method based on a chitosan-Prussian blue nanocomposite membrane potentiometric sensor was developed to determine amlodipine selectively[12]. Additional spectrophotometric methods for assessing AM in combination with other drugs have been published and proposed [13-16].

Hydrochlorothiazide (HCZ) , its scientific name is 2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide. It has the following chemical structure Fig. 2 The chemical formula of HCZ is $C_7H_8ClN_3O_4S_2$, and the molar mass = 297.7[1].

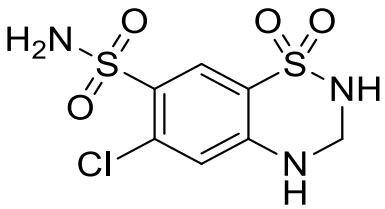

Figure 2. Structure of HCZ

It is an odorless white crystalline powder, slightly soluble in water, insoluble in chloroform, ether, and mineral acids, easily soluble in dimethylformamide, n-butylamine, and sodium hydroxide solution, and moderately soluble in methyl alcohol. HCZ is one of the most important thiazide diuretics that prevent water retention and is used, like other thiazide compounds, in treating high blood pressure alone or in combination with other antihypertensive compounds (ACE Inhibitors) and beta-blockers. It is also used to treat edema associated with heart failure, premenstrual syndrome, and preventive symptoms from kidney stones. It is effective for patients with diabetes insipidus and is also used sometimes in cases of high urinary calcium levels[17]. The literature review shows many methods for measuring HCZ in tablets and biological fluids, including direct spectrophotometric measurement at a wavelength of 272 nm[18]. The electrochemical method by differential pulsed voltammetry using a glassy carbon electrode was modified with multi-walled carbon nanotubes and gold nanoparticles[19]. HCZ was evaluated with AM and valsartan in combined dosage form using RP-HPLC methods [20, 21], and FTIR spectroscopic method was developed to determine valsartan and HCZ[22] simultaneously. The simultaneous evaluation of nebivolol hydrochloride and HCZ has been established based on the partial simultaneous equation [23]. A voltammetric sensor based on a carbon paste electrode modified with an AlV_3O_9/CNT nanocomposite was developed to determine AM and HCZ[24]. Different methods for synthesizing AgNPs can be found in the literature; one of them is the chemical method, which usually uses three main components, specifically metal precursors, reducing agents, and stabilizers/caps. Common reducing agents are borohydride, glycerin, and 2-mercaptoethanol[25] or plant extract-based reducing agents[26 - 28]. The proposed method, $AgNO_3$ is used as the metal precursor and the analyte (AM drug and HCZ drug containing N-H groups) as the

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reducing agent, and sodium lauryl sulfate (SLS) as a stabilizer. The formation of AgNPs is indicated by a single surface plasmon resonance absorption band formed by the collective vibration of electrons in silver nanoparticles resonating with light waves. When the frequency of the electromagnetic field matches the coherent velocity of the electrons, significant absorption occurs, which is the source of the visible color [29,30]. The surface plasmon resonance absorption bands in the reagent blank disappear due to the absence of AM and HCZ (reducing agent). Many chemical species, including metal ions, pharmaceutical compounds, and proteins, have been analyzed using AgNPs [31 - 34] sensors. In brief, the proposed method based on forming AgNPs is environmentally safe, simple, sensitive, and selective and requires no calibration to characterize colloidal suspended particlestates.

Materials and Methods

Experimental – Instrumentation

Absorption measurements were performed using a Shimadzu UV-1900 spectrophotometer with a 1 cm wide quartz cuvette. The material was weighed using a sensitive balance (GR-200).

Materials and Reagents

The chemicals used in this study are all very pure. Pure AM and HCZ were provided by the Awa Medica pharmaceutical company, in Hawler, Iraq.
Sodium lauryl Sulphate($NaC_{12}H_{25}SO_4$),(92.89%), Awamedica
Sodium hydroxide (NaOH), (97%), pellets reagent grade, Scharlau-Spain
Silver nitrate (AgNO3) (99.9%) ACS metals basis, Germany

Pharmaceutical Preparations

AM pharmaceutical formulations are:

1. Lofral, 10mg Amlodipine besylate, Atral/Portugal.
2. Amlong, 10mg Amlodipine besylate , Micro/India.
3. Amlodipine, 5mg Amlodipine besylate, Bristol laboratories /UK.
4. Amlodipine, 10mg Amlodipine mesilate monohydrate, accord/ UK.
5. Amlodipine, 5mg Amlodipine mesilate monohydrate, accord/UK.
6. Lowvasc, 5mg Amlodipine besylate , Hikama/Gordan.
7. Lofral, 5mg Amlodipine besylate, Acino/Switzerland.
8. Amlong, 5mg Amlodipine besylate, Micro/ India.

HCZ pharmaceutical formulations are:

1. Hydrochlorothiazide, 25mg, Borisovskiy/Belarus.
2. HydrochlorothiazideT&D Pharma ,25 mg, GmBH/Germany.
3. Genkort, 10 mg, Cankaya /Ankara.
4. Esidrex, 25 mg, Novartis/Switzerland.

Standard Solutions

Standard Drug Stock Solution

A standard stock solution containing (100 µg mL^{-1}) of each AM, and HCZ was prepared by dissolving 0.01 g of pure AM in distilled water, and HCZ in ethanol , and diluting to 100 mL in a volumetric flask.

General Procedures

Construction of Calibration Graphs

A series of 5 mL volumetric flasks were filled with different drug AM and HCZ concentrations. Then 0.1% stabilizer, 0.01 M silver nitrate solution, and 0.125 M NaOH solution were added. The volume was filled to 5 mL with distilled water and heated to 80°C in a water bath. After a sufficient heating time, the absorbance of the solution at 420 nm for HCZ-AgNP and 418 nm for AM-AgNP was measured against the reagent blank, and the calibration curve was plotted.

Sample Preparation

Ten tablets were weighed to prepare the AM sample solution, and the average weight was calculated. The tablets were ground into a fine powder. The weighed tablet powder was dissolved in distilled water, transferred to a volumetric flask, and then filled with distilled water. The clear solution was diluted with distilled water to obtain an AM solution within the concentration range tested.

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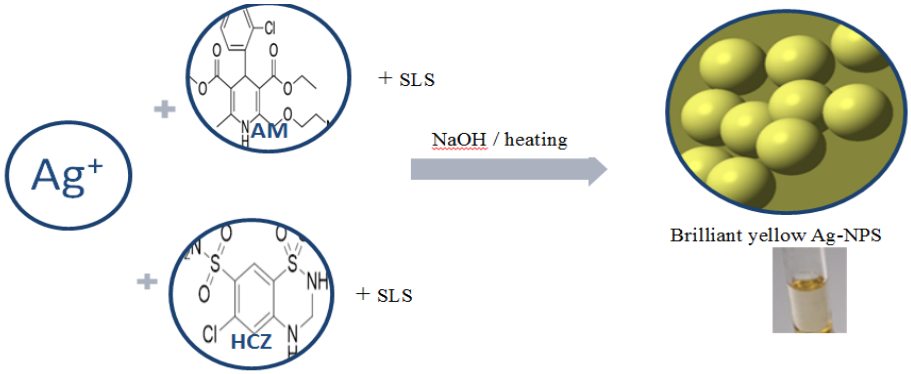
Ten tablets were weighed to prepare the HCZ sample solution, and the average weight was calculated. The tablets were ground into a fine powder. The weighed tablet powder was dissolved in a small amount of ethanol, followed by distilled water to achieve the desired volume. The solution was sonicated for 15 minutes before being centrifuged at 4000 rpm for 5 minutes. The clear supernatant was separated using a 0.45 M nylon syringe filter. The clear solution was diluted with distilled water to obtain HCZ solutions within the studied concentration ranges.

Results and Discussion

Optimization of the Reaction Conditions

Preliminary Test

In this study, drugs were used as effective reducing agents to reduce Ag^{+1} ions under alkaline conditions to form bright yellow silver nanoparticles, and surfactants were used to prevent aggregation of silver nanoparticles and increase its stability Scheme 1.



Scheme1.Formation of AgNPs by reduction of Ag^{+1} with AM and HCZ

The absorbance spectra of AgNPs was recorded between 300 - 700 nm, showing a well-known absorption band with maximum absorbance at 420 nm for AM- AgNPs and 405 nm for HCZ- AgNPs, which has been effectively used in the determination of the cited drugs Fig. 3.

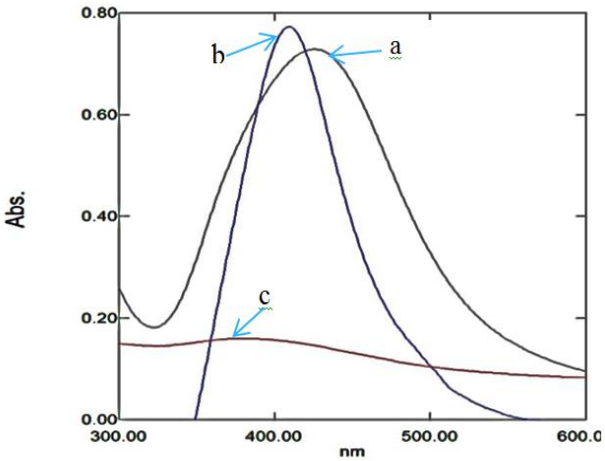


Figure 3. Absorption spectra of (a) 2 µg mL^{-1} of AM-AgNPs,(b)2 µg mL^{-1} HCZ-AgNPs , and (c) blank solution

Reaction Condition Optimization

Chemical conditions such as $AgNO_3$, surfactant, and NaOH concentrations, as well as temperature and heating time, were studied and modified to achieve the optimal silver plasmonic response.

Effect of NaOH Concentration

The intensity of absorption was affected by the concentration of sodium hydroxide. A wide range of NaOH molarities (0.1-0.2 M NaOH) were tested, with 0.125 M being the preferred molarity, and various volumes (0.1-1.3 ml) were tried. Maximum absorbance values for AM and HCZ were obtained with volumes of 0.9 ml NaOH and 0.6 ml NaOH, respectively , Fig. 4.

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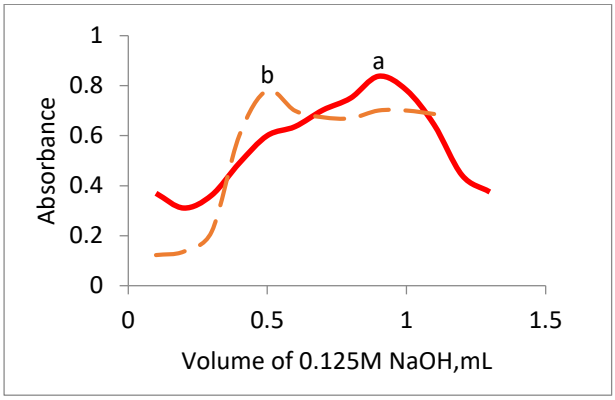

Figure 4. Effect of the volume of 0.125 M NaOH solution on the absorbance of a-AM-AgNPs, and b-HCZ- AgNPs

Effect of Silver Nitrate Concentration

Different concentrations of silver nitrate (0.01-0.02 M) were tested to determine the optimal concentration, which was found to be 0.01 M, from which various volumes ranging from 0.1 to 1.6 ml were examined, as shown in Fig. 5 For AM and HCZ, the maximum absorbance was measured at 1.2 ml and 0.7 ml $AgNO_3$, respectively.

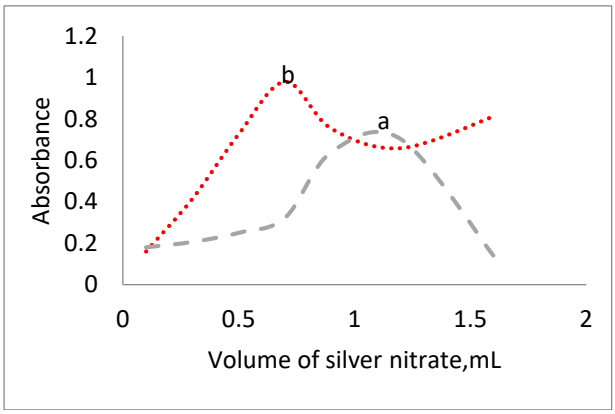

Figure 5. Effect of volume of 0.01M of silver nitrate solution on the absorbance a-AM-AgNPs , and b-HCZ-AgNPs

Effect of the Type and Concentration of the Stabilizer

To prevent the aggregation of silver nanoparticles, they must be stabilized. The stabilizers polyvinylpyrrolidone, sodium dodecyl sulfate, cetyltrimethylammonium bromide, SLS, and methylcellulose were tested. SLS was the best stabilizer to prevent the agglomeration of AgNPs. Different volumes of 0.1% SLS were tested, with the maximum absorption yield at 0.3 mL for AM and 0.35 mL for HCZ, Fig. 6.

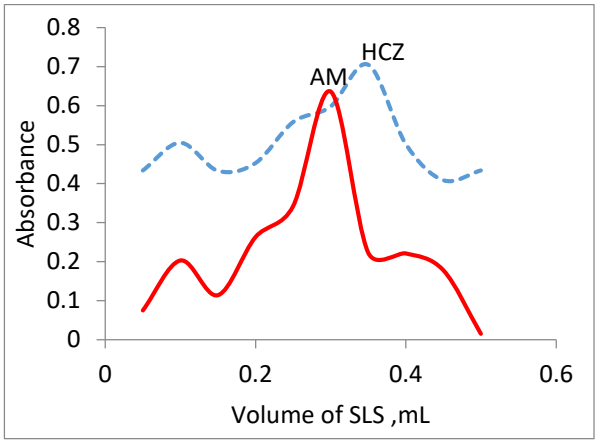

Figure 6. Effect of volume of 0.1% SLS solution on the absorbance of AgNPs

Effects of Temperature, Heating time and Stability

To accelerate the synthesis of AgNPs, it was necessary to heat the reaction flask. To obtain the highest color intensities for AM-AgNPs and HCZ-AgNPs, the solutions were heated in a water bath at 80°C and 90°C for 30 and 5 minutes, respectively, as shown in Figs. 7a and b. heating times of 30 and 5 minutes were sufficient to produce maximum absorption levels for AM-AgNPs and HCZ-AgNPs. The stability of the resulting nanoparticles was confirmed by measuring the absorbance over 65 minutes, Fig.7b.The absorbance remained constant, indicating that the generated nanoparticles were very stable.

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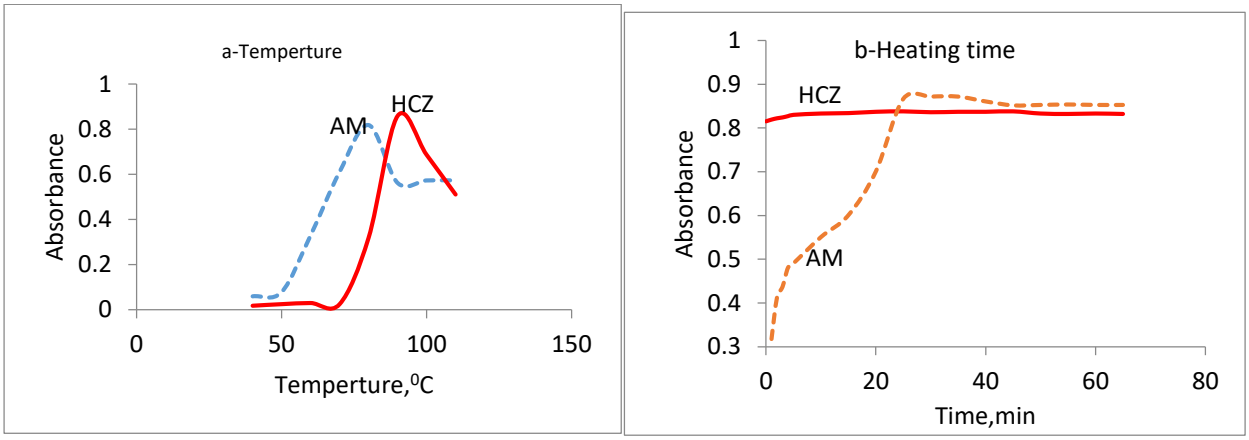


Figure7. Effect of a-temperature and b-heating time, and stability on the absorbance AM-AgNPs , and HCZ-AgNPs

Order of Addition

The order in which reagents were added can influence the rate of formation of silver nanoparticles. The most acceptable order for the current investigation was $AgNO_3$, SLS, drug, and NaOH.

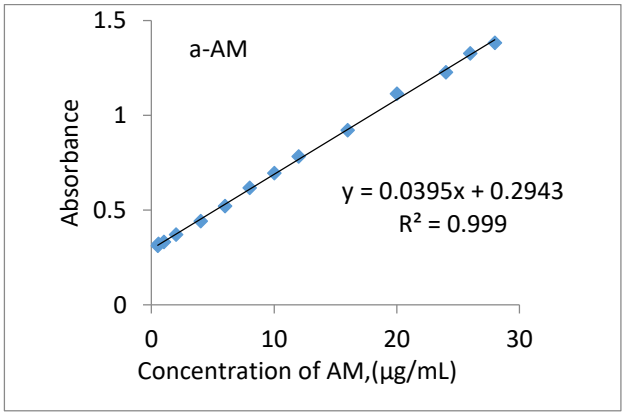
Method Validation

Linearity

Table 1. Optimum conditions for the determination of HCZ and AM by the formation of silver nanoparticles

Drug	Volume of NaOH (0.125 M)	Volume of Silver nitrate (0.01M)	Volume of SLS (0.1%)	Temperature ^{0}C	Time of reaction (min)	The Order of Addition
AM	0.9 ml	1.2 ml	0.30 ml	80	30	$AgNO_3$, SLS, AM, and NaOH
HCZ	0.6 ml	0.7 ml	0.35 ml	90	5	$AgNO_3$, SLS, HCZ, and NaOH

Calibration curves were plotted under the optimal experimental conditions, Table 1. Linearity ranges were (0.5-28 µg/mL) Fig .8a, and (0.80-6 µg/mL) Fig. 8b ,(each concentration was repeated three times) for AM and HCZ, respectively. The parameters of the regression equation were calculated in Table .2. The high value of the correlation coefficient and the small values of the intercepts, the standard deviation of the intercept, the standard deviation of the slope, and the standard error of the regression, indicate a good linearity of the method.



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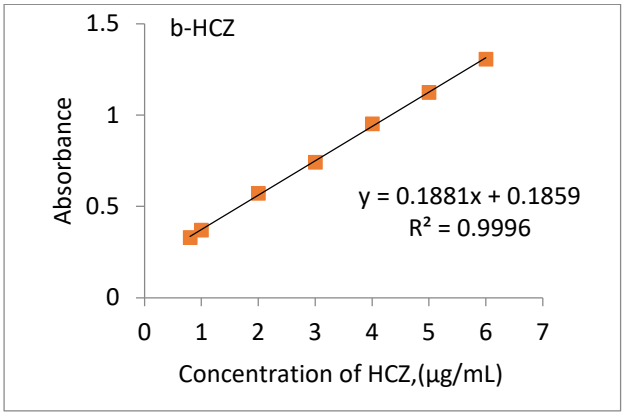

Figure 8. Calibration graphs of a- AM and b- HCZ

Limits of Detection (LOD) and Quantitation (LOQ)

To measure the method's sensitivity, the LOD and LOQ were calculated using the following formulas: LOD = 3.3 σ / S and LOQ = 10 σ / S, where the σ is the standard deviation of the intercept and S is the slope of the calibration curve. LOD and LOQ values varied from 0.442 to 1.336 for AM, and 0.128 and 0.388 µg/mL for HCZ, respectively Table .2. These low values demonstrate the suggested method's high sensitivity.

Table 2. Summary of quantitative parameters and statistical data for the determination of the HCZ, and AM with the proposed method

Parameter	AM	HCZ
Regression equation	y = 0.0395x + 0.2943	y = 0.1881x + 0.1859
linear range (µg mL^{-1})	0.5-28	0.8-6
Slope(mL/µg)	0.0395	0.1881
Standard deviation of slope (Sb)	0.0003	0.0020
Intercept	0.2943	0.1859
Standard deviation of intercept (Sa)	0.0052	0.0073
determination coefficients, r^2	0.9990	0.9996
correlation coefficients, r	0.9995	0.9997
Standard error of the regression S X/Y	0.0127	0.0098
Molar absorptivity, ε (L.mol-1.cm-1)	16130	55419
Sandal's sensitivity S (µg.cm-2 per 0.001 absorbance unit)	0.0252	0.0053
LOD (µg mL^{-1})	0.442	0.128
LOQ (µg mL^{-1})	1.336	0.388

Accuracy and Precision

The accuracy (percent recovery) and precision (percent RSD) of the current technique for measuring AM and HCZ were studied with three concentrations in six replicates. The results in Table 3 show that the proposed approach is very accurate and precise, with recovery % of 98.9-103.66 for AM and 98.59-99.41 for HCZ and low RSD values ranging from 0.060 to 3.430 for AM and 0.552 to 0.832 for HCZ respectively.

Table 3. Accuracy and precision

Drug	Concentration (µg mL^{-1})		Recovery (%)	E%	% RSD*
	Added	Found			
AM	2	1.978	98.90	1.100	3.430
	12	12.439	103.66	-3.665	0.210
	26	26.101	100.39	-0.390	0.060
HCZ	1	0.992	99.21	0.784	0.552
	3	2.957	98.59	1.400	0.832
	6	5.964	99.41	0.590	0.610

* Six repeated measurements.
RSD: Relative standard deviation, E: Error.

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Application of the Method

The amount of AM and HCZ in the pharmaceutical formulations was measured. The results are summarized in Tables 4 and 5. The table shows that the method is very accurate with recoveries of 94.16-102.10 for AM and 96.16-98.30 for HCZ, demonstrating the potential applicability of this method to determine AM and HCZ drugs in pharmaceutical products.

Table 4. Determination of AM in pharmaceutical formulations

	Commercial name	Chemical name	Content mg	Found mg	Recovery (%)*
1	Lofral	Amlodipine besylate	10	9.553	95.53
2	Amlong	Amlodipine besylate	10	10.210	102.10
3	Amlodipine	Amlodipine besylate	5	4.708	94.16
4	Lowvasc	Amlodipine besylate	5	5.040	100.80
5	Lofral	Amlodipine besylate	5	4.906	98.12
6	Amlong	Amlodipine besylate	5	4.850	97.00

*Average of four repeated measurements.

Table 5. Determination of HCZ in pharmaceutical preparations

	Commercial name	Chemical name	Content mg	Found mg	Recovery (%)*
1	Hydrochlorothiazide	Hydrochlorothiazide	25	24.577	98.30
2	HydrochlorothiazideT&D Pharma	Hydrochlorothiazide	25	24.517	98.06
3	Genkort	Hydrochlorothiazide	10	9.616	96.16
4	Esidrex	Hydrochlorothiazide	25	24.249	96.99

*Average of four repeated measurements.

Conclusion

A straightforward and accurate spectrophotometric method has been proposed to determine AM and HCZ based on the redox reaction supported by the drugs' reduction of Ag^{+1} to form silver nanoparticles (AgNPs). These drugs have an N-H group that can be oxidized in an alkaline environment by losing H to generate an anion, which is easier to reduce Ag^{+1} to Ag^{0} and produce AgNPs. AgNPs are particularly brilliant yellow and have a maximum absorbance of 418 nm for AM and 420 nm for HCZ in the presence of SLS as a stabilizer. The method was simple, accurate, precise, sensitive, safe, and successfully applied to determine each AM and HCZ drug in its pure form and pharmaceutical preparation.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Hawler Medical University.
- Ethics statement:
  No animal studies are present in the manuscript.
  No human studies are present in the manuscript.
  No potentially identified images or data are present at the manuscript.

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Authors' Contribution Statement

L.S.O. designed and directed the project and wrote the paper with input from all authors, and R.J.A., N.N.H., and A.G.D. performed the measurements and calculations.

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الاستفادة من رنين البلازمون السطحي الموضعي لجسيمات الفضة النانوية لتقدير الطيف الضوئي للأمولوديبين وهيدروكلوروثيازيد

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الخلاصة

أمولوديبين هو مانع قوي وطويل المفعول لقنوات الكالسيوم. يمكن للمرضى الذين يعانون من ارتفاع ضغط الدم تناوله لعلاج ارتفاع ضغط الدم والذبحة الصدرية وتقليل مخاطر الإصابة بالسكتة الدماغية. يستخدم مدر للبول يسمى هيدروكلوروثيازيد لعلاج الوذمة وارتفاع ضغط الدم. بالإضافة إلى ذلك ، يتم استخدامه لعلاج بعض أنواع مرض السكري وارتفاع السكر في الدم ونقص بوتاسيوم الدم. تتوفر هذه الأدوية على نطاق واسع في السوق وغالبًا ما يتم تناولها عن طريق الفم. وبالتالي ، تم إنشاء طريقة سريعة ودقيقة وآمنة بيئيًا وغير مكلفة لقياس أمولوديبين وهيدروكلوروثيازيد والتحقق منها. كانت قدرة الدواء على اختزال Ag^{+1} إلى Ag^{0} مباشرة وإنشاء جزيئات الفضة النانوية (Ag NPs)

في وجود كبريتات لوريل الصوديوم كمثبت أساس الطريقة. ظهر صدى كبير للبلازمون السطحي للجسيمات النانوية المركبة عند 418 نانومتر و 410 نانومتر ، والتي تم استخدامها للكشف الطيفي الكمي للأمولوديبين وهيدروكلوروثيازيد. مع حدود الكشف عن 0.442 و 0.128 ميكروغرام /مل ، كانت نطاقات التركيز الخطي للأمولوديبين وهيدروكلوروثيازيد 0.5 - 28 و 0.8 - 6 ميكروغرام /مل على التوالي. تم استخدام الطريقة المقترحة لتقييم أشكال الجرعات التجارية التي تحتوي على الأدوية المدروسة بنجاح.

الكلمات المفتاحية: أمولوديبين، هيدروكلوروثيازيد، جسيمات الفضة النانوية، رنين سطح البلازمون، قياس الطيف الضوئي.