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Determination of Amlodipine by Spectrophotometric Analysis involving Diazotization and Coupling with Sulfanilic acid

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Abstract

The research aims to develop and evaluate a spectrophotometric method to quantify amlodipine in pharmaceutical formulations. The method demonstrated high sensitivity with a molar absorptivity of 69,744.87 $L \cdot mol^{-1} \cdot cm^{-1}$ and a Sandell's sensitivity of 8.51×10^{-6} $\mu g \cdot cm^{-2}$. Linearity was observed in the range of 1.0-10 µg/ml, with a correlation coefficient (R^2) of 0.9984. The detection limit (LOD) was calculated as 0.0987 $\mu g \cdot ml^{-1}$ and the quantitative limit (LOQ) was found to be 0.2991 $\mu g \cdot ml^{-1}$. Precision and accuracy were determined by testing three different concentrations of amlodipine, resulting in relative standard deviations (RSD%) ranging from 0.21-1.01% and relative errors (RE%) between (-2 to 1%). The method was successfully applied to commercial pharmaceutical products, demonstrating recovery range of 101.42-103.40%. This study provides a reliable and efficient analytical approach for assessing amlodipine in various pharmaceutical formulations.

Introduction:

Amlodipine identified as 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5 pyridine, dicarboxylic acid 3-ethyl 5-methyl ester, is also referred to as 'Fig. 1, [1]. It is a long-acting lipophilic agent and a third-generation calcium channel blocker (CCB) that specifically blocks calcium, ion influx through cell membranes.Vascular smooth muscle cells and cardiac muscle cells work together to lower peripheral vascular resistance (PVR) and decrease blood pressure. Amlodipine is effective in treating high blood pressure (BP)/HTN and angina[2].

The link between stroke and, myocardial infarction (MI) has not been proven yet[3]. It can also be utilized for dilated cardiomyopathy and shows, improvements in plasma and myocardial catecholamines while significantly decreasing calcium deposition [4].

Studies have demonstrated, that Amlodipine, a dihydropyridine CCB, can cause, apoptosis, leading to cell cycle arrest and inhibiting the growth of cancer cells [5]. Amlodipine

is useful for treating, unstable (Prinzmetal's) angina because, it causes vasodilation in the coronary arteries, allowing, more oxygen delivery to the heart [6].

Amlodipine is a light-sensitive , medication that needs to be shielded from light [7]. Several analytical techniques, such as high-performance thin layer liquid chromatography, were utilized to estimate the amount of ADB[8]. Gas chromatography connected with [9].

Additionally mass spectrometry is used [10]. Fluorimetry is mentioned in reference [11]. Spectrophotometry is one of the most commonly used scientific techniques in pharmaceutical analysis serving as a quantitative rational approach. It offers tangible economic benefits compared to approach [12].

The method aims to create a sensitive and reliable spectrophotometric approach for quantifying amlodipine in pharmaceutical , formulations. It utilizes diazotization and coupling with sulfanilic acid in an alkaline solution producing an orange azo dye with an absorption peak at 490 nm. The method offers high precision, accuracy, and linearity, making it suitable for ,quality control and analysis of commercial pharmaceutical products.

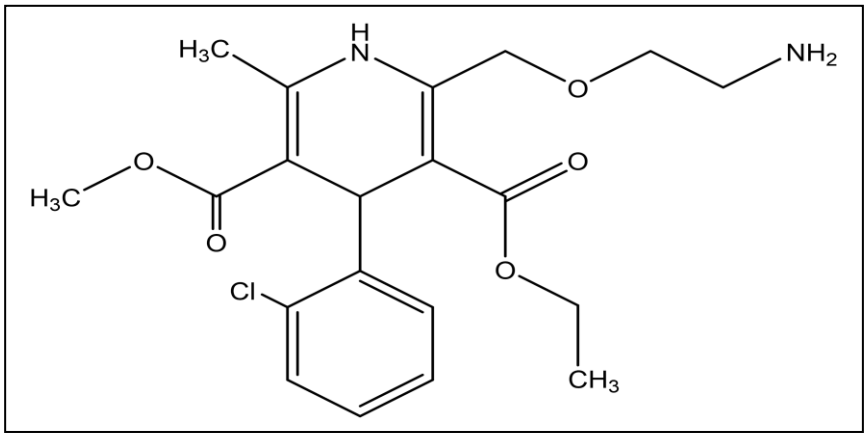

Fig. 1: The chemical structure of the amlodipine

Experimental:

Instrumentation part

Measurements of spectral and absorbance were conducted with the Jasco V-650 double beam spectrophotometer and cm^{-1} plastic cells, while weights were determined using the four-ranked electrical scale Model: AS 220/C/1.

Used chemical reagent and materials

All synthetics utilized are of the greatest virtue that anyone could hope to find.

Amlodipine solution (100 µg.ml^{-1}):

A specific amount of Amlodipine (0.01 g) was dissolved in a defined volume of distilled water, and the solution was then made up to 100 ml using a volumetric flask with distilled water.

hydrochloric acid solution (2 M):

Made by mixing 17.2 ml of concentrated hydrochloric acid with a concentration of 11.64 M with 100 ml of distilled water.

Sodium nitrite solution (1.0 %):

One g of sodium nitrite was mixed with a defined volume of distilled water and then the mixture was topped up with distilled water in a 100 ml volumetric flask [13].

Sulfanilic acid solution (1.0 %):

One g of sulfanilic acid was dissolved in a designated amount of distilled water and the solution was then topped up with distilled water in a 100 ml volumetric flask [13].

Sodium hydroxide solution (2 M):

Eight g of Sodium hydroxide were dissolved in a certain amount of distilled water followed by dilution with additional distilled water in a 100 ml volumetric flask.

Pharmaceutical solutions (100 µg.ml^{-1}) :

Twenty tablets of amlong and amaday were accurately weighed and then crushed into finely powdered form. 5 mg of amlodipine was dissolved in 25 ml of distilled water and solvent mixture then filtered using filter paper. Finally, the solution was ,adjusted to a volume of 50 ml with the same solvent mixture [4].

Results and Discussion

During initial tests, it was observed that when 1.5 ml of 1% sodium nitrite solution was combined with 0.5 ml of 2 M hydrochloric acid and shaken occasionally. Next, 1.5 ml of a 1% solution of Sulfanilic acid should be added with intermittent shaking for (5 minutes) at room temperature [13,14]. Following the addition of 100 µg.ml^{-1} amlodipine solution and 1.0 ml of 2 M sodium hydroxide solution, the final mixture in a 10 ml bottle was adjusted with distilled water to the mark. The absorption spectrum of the colored solution was then recorded, displaying peak absorption at 490 nm compared to Fig. 2,.

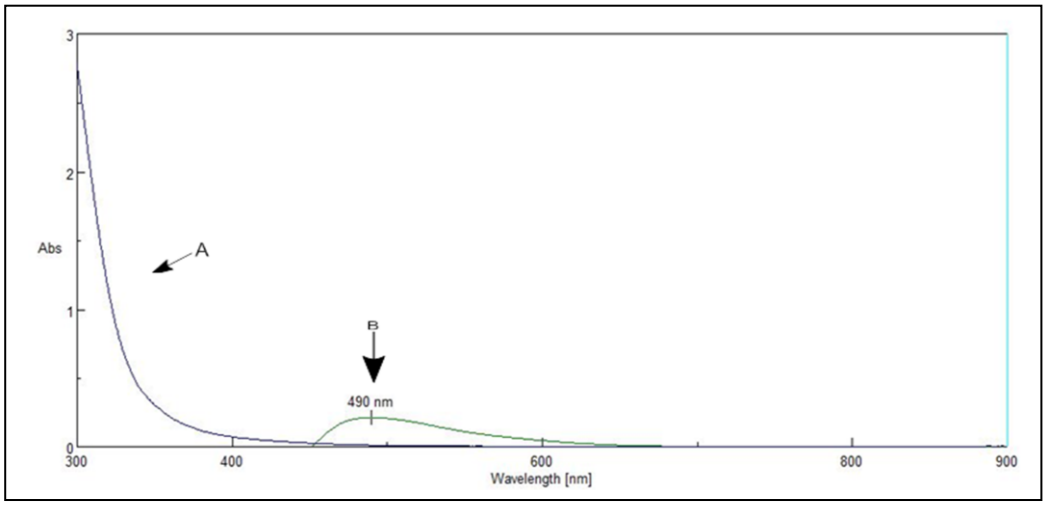

Fig. 2: (A) blank against distilled water
(B) Absorbtion spectrum of amlodipine product against blank

Spectrophotometric determinate

Influence of the volume of sodium nitrite

The influence of 1.0% $NaNO_2$ was examined using varying volumes, and findings from Table 1 demonstrated peak absorbance with the addition of 1 ml of 1.0% sodiunitrate.

Table 1: Influence of varying $NaNO_2$ levels.

Volume ml of 1.0% $NaNO_2$	Absorption
0.5	0.263
0.8	0.340
1	0.427
1.5	0.211
2	0.206

Influence of volume of hydrochloric acid

Research has been conducted on the influence of the volume of hydrochloric acid. The ideal volume was found to be 0.3 ml of 2 M hydrochloric acid solution as shown in Table 2, Where the acid works to create a diazonium salt by inserting a proton and withdrawing the water molecule

Table 2: Displays the influence of the volume of hydrochloric acid.

Volume ml of 2 M HCl	Absorption
0.1	0.445
0.3	0.514
0.5	0.427
0.8	0.415
1	0.382

Influence of the volume of sulfanilic acid

Various amounts of a 1.0% solution of sulfanilic acid were used with all other factors remaining consistent. An optimal volume of 2 ml of the reagent solution was determined based on Table 3.

Table 3: Displays the influence of varying amounts of sulfanilic acid.

volume ml of 1.0 % sulfanilic acid	Absorption
1	0.368
1.5	0.415
2	0.611
2.5	0.377
3	0.080

Influence of sodium hydroxide

The volume of NaOH (2 M) was examined, and 0.3 ml resulted in the highest intensity of the azo dye, so it was chosen for further experiments as seen in Table 4.

Table 4: Demonstrates the influence of varying amounts of sodium hydroxide.

Volume ml of 2 M NaOH	Absorption
0.1	0.318
0.3	0.855
0.5	0.611
0.8	0.425
1	0.377

Influence of the times

Once the best conditions were established, it was determined that the ideal time needed for the diazonium salt formation is five minutes as shown in Table 5, while the reaction time between the reagent and diazonium salt should be zero minutes as indicated in Table 6. Furthermore, the analysis of the final reaction's absorbance demonstrates that the highest absorbance is achieved 20 minutes after initiation and stays constant for at least 24 hours as shown in Table 7.

Table 5: Illustrates how the time influence the creation of the diazonium salt.

Time (minute)	Absorption
0	0.298
3	0.318
5	0.855
8	0.513
10	0.425

Table 6: Effect of time on the interaction between the detector and the diazonium salt.

Time (minute)	Absorption
0	0.855
5	0.527
10	0.253

Table 7: Influence of duration on the durability of pigmented azo dye.

Time (minute)	Absorption
0	1.274
10	1.359
20	1.432
30	1.421
40	1.430
50	1.429

Suggested steps and graph for calibration

Based on the best conditions found, various amounts ranging from 0.1 to 1 ml of a standard solution with a concentration of 100 µg.ml^{-1} of amlodipine were transferred into a set of 10 ml Volumetric bottles. Solutions with varying concentrations were then prepared, the absorbance was measured at 490 nm, and the results were recorded. Combine 1.0 ml of 1.0% sodium nitrite solution and 0.3 ml of 2 M hydrochloric acid in a 10 ml Volumetric bottle, shaking occasionally. Then, add 2.0 ml of 1.0% sulfanilic acid solution and let it sit for five minutes at room temperature. Next, various drug solution volumes containing 10-100 µg are mixed with 0.3 ml of 2 M sodium hydroxide and filled up with distilled water to the mark. The absorbance value is measured at 490 nm against the reference solution in 'Fig. 3, Approach is very high sensitive through molar absorpitivity 69744.87 L.mol^{-1}.cm^{-1}, and Sandell's sensitivity 8.51×10-6 µg.cm^{-2}.

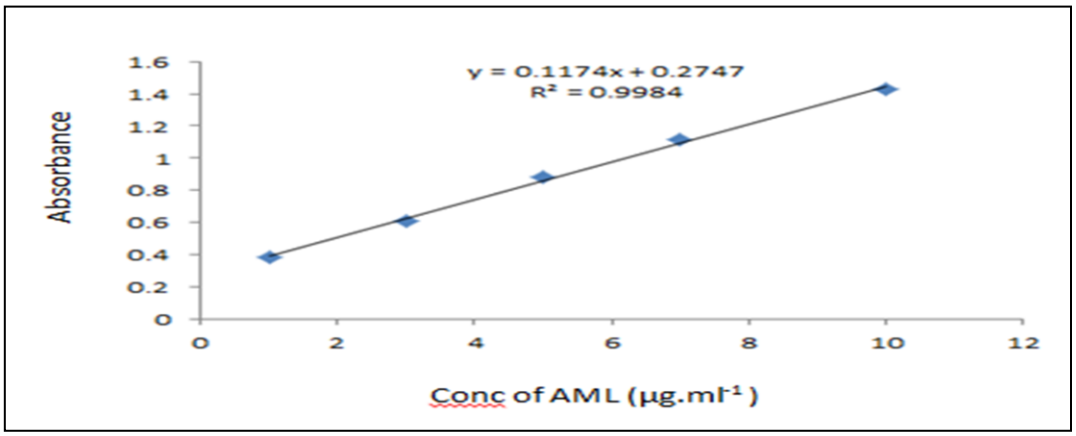

Fig. 3: Graph showing the calibration of AML measurements.

Detection Limit and Quantitative Limit

The detection limit and the quantitative limit have been calculated the slightest concentration 1 µg.ml^{-1} in the calibration curve at the optimum misfortune (because the blank is colorless) as shown in the Table 8 [15].

Table 8: Detection Limit and Quantitative Limit.

Concentration µg.ml^{-1}	Slope	SD	LOD µg.ml^{-1}	LOQ µg.ml^{-1}
1	0.1174	0.0035	0.0987	0.2991

Accuracy and Precision:

Three varying levels of concentration from 2 to 6 μg.ml^{-1} were selected for the calibration curve. Each concentration was tested three times to determine the Precision (RSD%) and accuracy (RE%) of the approach. The outcomes can be seen in Table 9.

Table 9. The approach precision and accuracy in determining amlodipine

Concentration Sample (μg.ml^{-1})		Rec%	RE%	SD	RSD%
Taken	Found				
2	1.98	99	1	0.0051	1.0158
4	4.08	102	-2	0.0030	0.4048
6	6.08	101.33	-1.33	0.0020	0.2105

Suggested reaction mechanism:

The proposed reaction mechanical clarifies as in the figure 'Fig. 4, [14] .

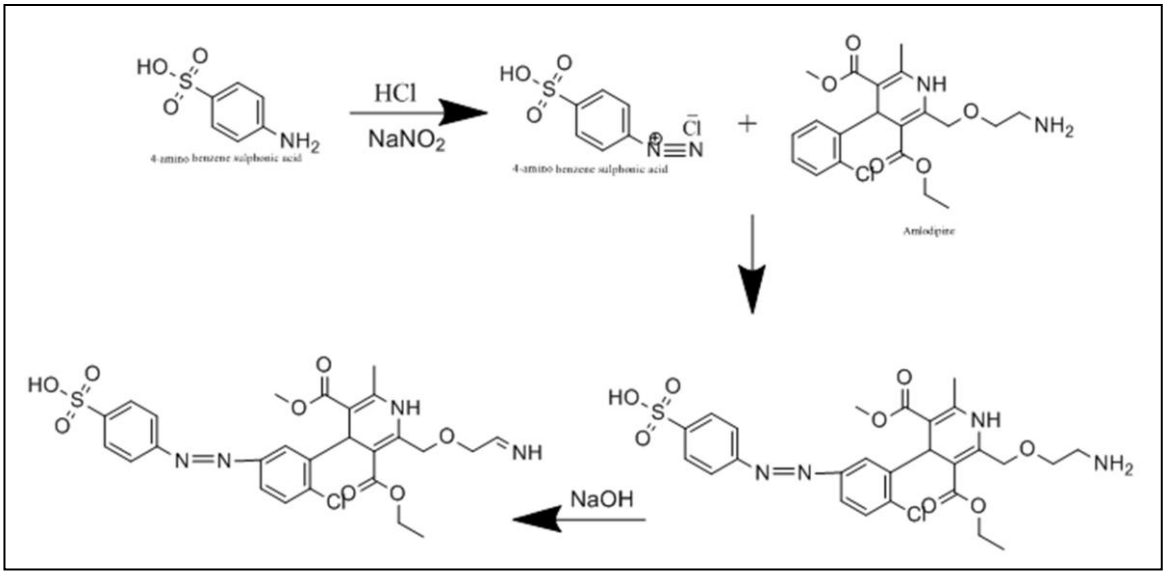

Fig. 4: Suggested reaction mechanism.

Application for analysis

The proposed approach used to analyze amlodipine within various pharmaceutical products. After following the suggested approach, a favorable outcome was achieved, as demonstrated in Table 10.

Table 10: The analytical application of the proposed approach.

Formulation name	Concentration Sample (μg.ml^{-1})		Recovery %	RE%	SD	RSD%
	Taken	Found				
Amlong 5 mg	5	5.11	102.20	-2.20	0.0049	0.5639
	7	7.10	101.42	-1.42	0.0041	0.3755
Amaday 5 mg	5	5.17	103.40	-3.40	0.0030	0.3465
	7	7.23	103.28	-3.28	0.0050	0.4448

Approach comparison

'Table 11 displays the comparison of analytical parameters for amlodipine determination between the proposed approach and the literature approach.

Table 11: Comparison of approach

Analytical parameters	Proposed approach	Literature approach [14]	
Reagent	Sulfanilic acid	p-nitroaniline	Crystal violet dye
Medium	alkaline	acidic	acidic
λmax (nm)	490	368	592
Range of Beer's law (μg .ml^{-1})	1-10	10-200	5-92.5
Molar absorptivity (L.mol^{-1}.cm^{-1})	69744.87	3.42×105	2.13×105
Product's color	orange	yallow	blue
Utilization of the technique	Medicinal drugs getting ready	Medicinal drugs getting ready	Medicinal drugs getting ready

Conclusions

The new spectrophotometric approach provides a simple and effective way to detect amlodipine in pharmaceutical products. Through the process of diazotization and coupling with sulfanilic acid in a basic solution, a unique orange water-soluble azo-dye is produced, allowing for straightforward identification. The approach follows Beer's law and shows strong sensitivity across the designated range of concentrations. In general it offers a hopeful answer for regular evaluation of amlodipine content, guaranteeing product quality and adherence to regulatory criteria.

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تقدير الأمليوديبين عن طريق التحليل الطيفي الذي يتضمن الازوتة والاقتران بحامض السلفانيليك

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معلومات المؤلف
الايميل:
الموبايل:

الخلاصة:
يهدف البحث إلى تطوير وتقييم طريقة طيفية لقياس كمية الأمليوديبين في المستحضرات الصيدلانية. أظهرت الطريقة حساسية عالية مع امتصاص مولاري 69,744.87 لتر•مول$^{-1}$•سم$^{-1}$ وحساسية ساندل، 8.51×10^{-6} ميكروغرام•سم$^{-2}$. ضمن حدود الخطية 10-1.0 ميكروغرام/مل، مع معامل ارتباط (R^2) قدره 0.9984. تم حساب حد الكشف (LOD) 0.0987 ميكروجرام•مل$^{-1}$، والحد الكمي (LOQ) 0.2991 ميكروجرام•مل$^{-1}$. تم تحديد الدقة والضبط من خلال اختبار ثلاثة تراكيز مختلفة من الأمليوديبين، مما أدى إلى انحرافات معيارية نسبية (RSD٪) تتراوح بين 1.01-0.21٪ وأخطاء نسبية (RE٪) بين (2- إلى 1٪). تم تطبيق الطريقة بنجاح على المنتجات الصيدلانية التجارية، مما يدل على الاسترجاعية، في نطاق 103.40-101.42%. توفر هذه الدراسة منهجًا تحليليًا موثوقًا وفعالًا لتقييم الاملوديبين في التركيبات الصيدلانية المختلفة.