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Spectrophotometric methods for estimation of amlodipine besylate in pure form and in it's pharmaceutical formulations

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***Abstract*---**Two unexpensive, sensitive, reproducible UV-spectrophotometric methods were developed for the determination of Amlodipine Besylate (ADB) in pure form and in pharmaceutical dosage forms. The first method was using Crystal violet dye (CV) as reagent (Tris (4-dimethyl amino) phenyl) methylium chloride). This method relied on oxidizing (ADB) in acidic medium in presence of an oxidizing agent of potassium bromide: potassium bromate ($KBr:KBrO_3$) to form a blue colored product of absorbance at λ_{max} 592 nm, It obeys the Beer's law in a concentrations range of (5-92.5) µg/ml with a molar absorptivity (2.13×10^5) L/mole.cm and the limit of detection is (0.01) µg/ml. The second method includes the reduction reagent p-nitroaniline using a nitrogenous agent ($NaNO_2$) in an acidic medium followed coupling reaction between (ADB) and p-nitroaniline to form yallow product has absorbance at λ_{max} 368 nm, It obeys the Beer's law in a concentrations range of (10-200) µg/ml with a molar absorptivity (3.42×10^5) L/mole.cm and the limit of quantification is found to be (0.01) µg/ml. The tow proposed methods are successfully applied for measuring the quantity of (ADB) in pure form and in tablets formulations.

***Keywords*---**Spectrophotometry, Amlodipine besylate, Crystal violet, p-nitroaniline.

Introduction

Amlodipine Besylate (ADB) chemically known as 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl),1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid and 3-ethyl-5-methyl ester[1], (Figure 1)[2], (ADB) is an important a calcium channel blocker with vasodilatory a ctivity similar to those of nifedipine, is mainly used in antihypertensive, antianginal and antiarrhythmic activities[3]. The main effects of this drug are confined with peripheral and coronary vasodilator properties. Therefore, the analysis of its forms is very important [4]. Various analytical methods were used to estimate of (ADB) including, high-performance thin layer liquid chromatography [5,6], gas chromatography coupled with mass spectrometry [7], high performance liquid chromatography [8-13] and fluorimetry[14]. Spectrophotometry as a quantitative analytical method still belongs to the most frequently used analytical techniques in pharmaceutical analysis. It provides practical and significant economic advantages over methods [15,16].

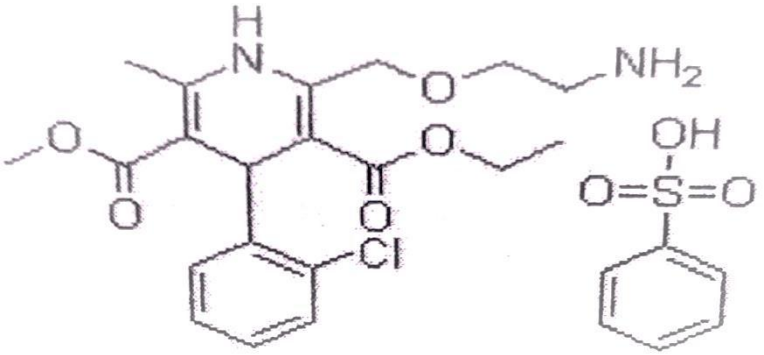


Fig. (1): Chemical structure of (ADB)

The purpose of the study

The aim of the research to develop a quick, economical and simple spectrophotometric methods for determination of (ADB) by using reagent Crystal violet dye (CV) with oxidizing agent in an acidic medium and reduction reagent p-nitroaniline in an acidic medium followed coupling reaction between ADB and p-nitroaniline in a basic medium.

Apparatus

Spectrophotometeic analysis was carried out by using a double beam UV-Vis spectrophotometer (shimadzu UV Spectrophotometer (UV-1800)) with 1 cm matched quartz cell's, T90 UV-Vis. Spectrophotometer double beam from PG instruments LTD, with 1cm quartz cell's, sensitive balance type (KERN ABS 120-4N) and oven from (Memmert, Schutzart DIN 40050-Ip20**).**

Materials

Amlodipine Besylate 99% from (SDI Samarra. Iraq). Crystal Violet 99% from (Merck), Potassium bromate 98% from (Merck), Potassium bromide 99% from (Merck), Hydrochloric acid 36% from (Thomas baker), Sodium nitrite 98% from

(Scharlau), Ethanol 99.9% from (Scharlau)**,** NH_4OH from (Fluka), p- nitroaniline 99% from (SDI Samarra. Iraq).

Solutions

- Amlodipine Besylate (1000 µg/ml): dissolve (0.1 gm) of (ADB) in (100 ml) of absolute ethanol.
- Crystal Violet ($3x10^{-5}$ M): dissolve (0.0012 gm) in (100 ml) of distilled water.
- The oxidizing agent (KBr-$KBrO_3$): prepared by dissolving (0.1 gm) of Potassium bromite and (1 gm) of Potassium bromade in (100 ml) of distilled water, (2.5 ml) of the mixture are diluted by distilled water in (100 ml) of volumetric flask.
- Hydrochloric acid solution (1.0 M): Prepared by diluting (8.6 ml) of concentrated acid (11.64 M) to (100 ml) distilled water and (2.0 M) of HCl Prepared by diluting (8.6 ml) of concentrated acid (11.64 M) to (50 ml) distilled water.
- NH_4OH (1 M): prepared by diluting (7.7 ml) from (6.493 M) in (50 ml) distilled water.
- $NaNO_2$ (1×10^{-3}M): prepared by dissolving (0.0069 gm) in (100 ml) of distilled water.
- P-nitroaniline (1×10^{-3} M): dissolved (0.0138 gm) in (100 ml) of absolute ethanol.

Procedures

Determination of (ADB) by Crystal Violet (CV) dye:

After initial testing, optimal conditions were obtained, transferring (1.0 ml) of (250 µg/ml) (ADB) to a 10 ml volumetric flask, then added (1 ml) of KBr - $KBrO_3$. After 10 min., added (0.5 ml) of (1.0 M) HCl acid. After 10 min., with constant shaking, added (3.0 ml) of (3×10^{-5} M) from (CV), and after 5 min., the volume is supplemented with ethanol to (10 ml), giving the highest absorption of the resulting compound 0.836 at room temperature at 592 nm**.**

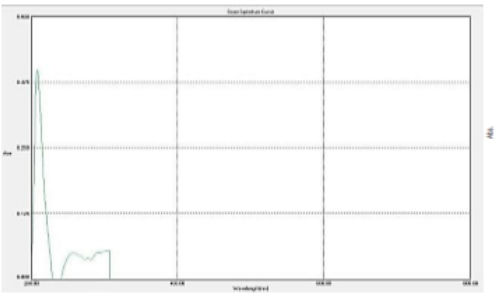


Fig. (2): Absorption spectrum of blank against ethanol

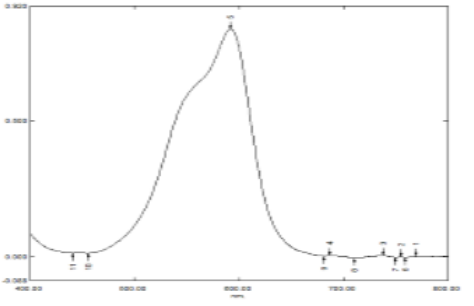


Fig. (3): Absorption spectrum of (ADB) product against blank

Application of proposed method

Ten tablets were weighed and average weights was computed. These tablets were grinded into exact powder. An precisely weighed amount of powder was transferred into a beaker and they were shaken with 50 ml solvent and filtered the filtrates and the washings were collected in a 100 ml volumetric flask this

filtrate and the washing was diluted up to the mark with solvent to obtain final concentration as 250 µg/ml the suggested methods were successfully implemented for the determination of (ADB) in various commercial tablets.

Results and Discussion

"Optimal Conditions"
Effect of stability time

The stability of the product formed from the interaction of the drug with the reagent is important in order to know the period of time during which the formed product can remain stable. From the experiments that were conducted, it was observed that the absorption values remained stable until the next day (24) hours, which is an excellent time for carring out measurements.

Effect of (CV) dye volumes

Figure (4) shows the effect of adding different volumes of the Crystal violet dye on the absorption of the product. The best added volume was (3 ml).

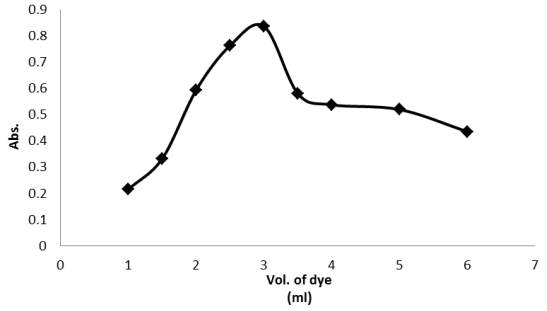


Fig. (4): Effect of dye volumes on the product

Effect of acid volumes

Increasing and different volume of HCl were used at a concentration of (1.0 M), to know which volume gives the best absorption, it was found that the best volume is (0.5 ml), as shown in figure (5).

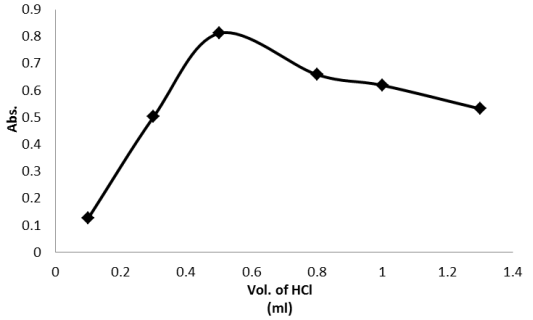


Fig. (5): Effect of HCl volumes of on the product

Effect of different acids

It include weak and strong acids (HCl, H_2SO_4, HNO_3 and CH_3COOH) of a concentration of (1.0 M), as well as, the same volume added (0.5 ml), to find which acid have the best absorption of product formed, The results showed that HCl acid is the best product, as shown in table 1.

Table (1)
Effect of different acids on the product

Acid	Absorbance
H_2SO_4	0.474
HNO_3	0.415
HCl	0.836
CH_3COOH	0.015

Effect of oxidizing agent volumes

Increasing volumes of (KBr-$KBrO_3$) were added to find its effect on absorption, the best added volume of oxidizing agent is (1 ml), as shown in figure (6).

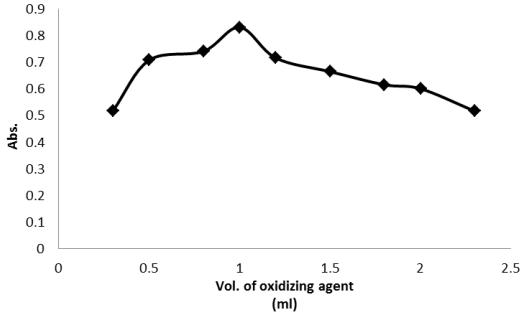


Fig. (6): Effect of (KBr-$KBrO_3$) Volumes

Effect of Additives
As shown in the table (2) there is no effect of additives on absorption values.

Table (2)
Effect of additives

Interference	Added con. µg/ml	%RE	Added con. µg/ml	%RE
Cellulose	50	4.19	100	2.75
Mognisum tearate	50	3.59	100	-0.72
Sodium carbonate	50	3.59	100	-1.54
Calcium carbonate	50	2.75	100	2.63
Mognisum carbonate	50	3.70	100	2.14

Calibration curve

The calibration curve for (ADB) with Crystal violet as a reagent, showed a linearity at concentrations range of (5-92.5) µg/ml, as shown in figure 7.

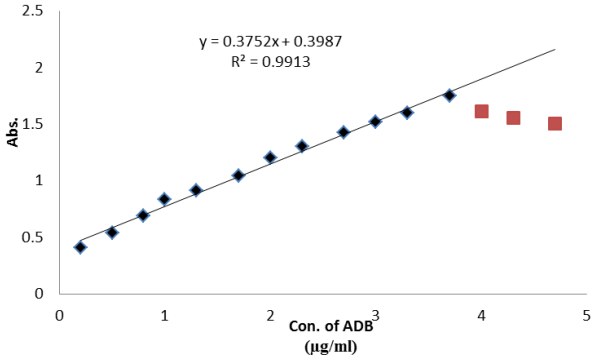


Fig.(7): Calibration curve of (ADB-CV) product

The characteristics of ealibration curve are shown in (table 3).

Table (3)
Characteristics of the Calibration curve for spectrophotometric determination of (ADB) product

Parmeters	ESO
$\lambda_{max.}$ (nm)	592
Beer's law (µg\ml)	(5 – 92.5)
Molar absorptivity (L\mol.cm)	2.13×10^5
Correlation coefficient (r)	0.9956
Limit of Detection (µg\ml)	0.01
Slope	0.3752
Intercept	0.3987
%RSD	0.283

Application of the proposed methods

In (Table 4), the results of determination of (ADB) in the pharmaceutical preparations (as tablet).

Table (4)
Determination of (ADB) (as tablet)

Pharmaceutical preparation	Content (µg/ml) declared	Found (µg/ml) by proposed method	%Recovery
Amlodipine (Amlong) Micro	20	19.84	99.20
	32.5	31.8	97.85
	50	50.76	101.52

Amlodipine (Accord) UK	20	20.7	100.36
	32.5	32.9	101.23
	50	49.62	99.24

The (stoichiometry) of the product
(Equation of the resulting product)

Under optimal conditions, the equivalence of the product interactions between (ADB), were studied with the reagent (CV) by mole ratio (at initial concentration 3×10^{-5} M), and continuous variations (at initial concentration 3×10^{-5} M) method. The equivalence between the reagent and this drug was 1:1 (Figures 8,9)

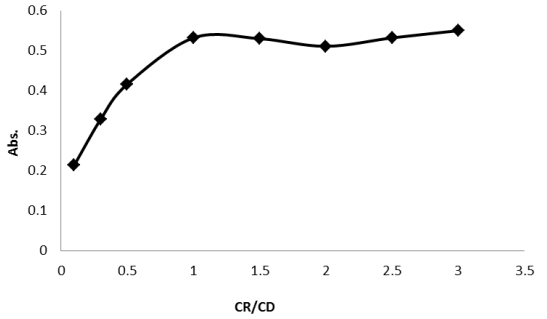


Fig.(8):Mole-ratio method of (ADB)

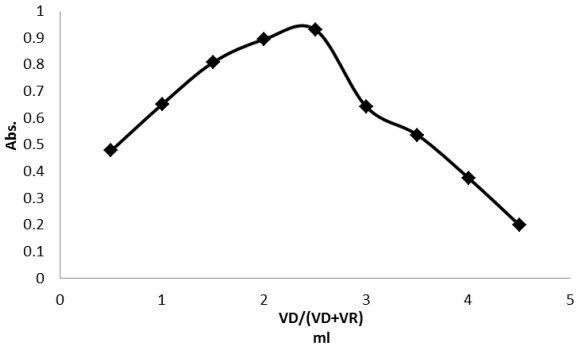
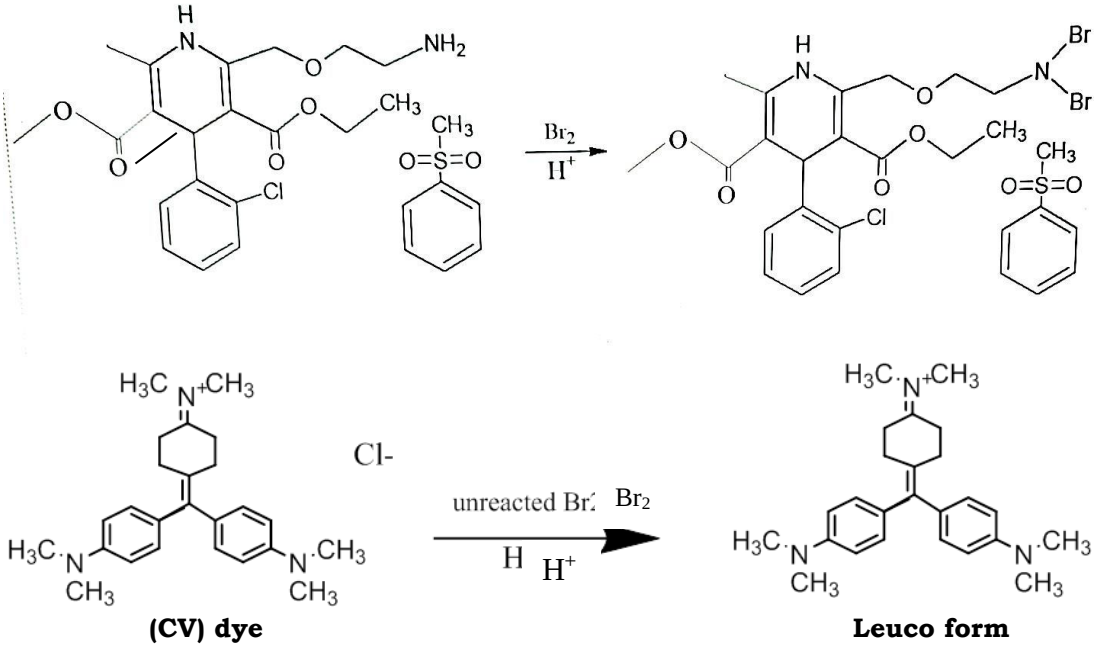


Fig.(9):Continuous variations method of (ADB)

Suggested interactions

The proposed reaction can be based on how the (ADB) drug is oxidized by ($KBr:KBrO_3$)[17] and reduced (CV) dye[18]:

$BrO_3^- + 5Br^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$



(II) Determination of (ADB) by p-nitroaniline

After initial tests, the optimum conditions were reached by transfer 1ml of P-nitroaniline (1×10^{-3} M) to a 10 ml volumetric flask, then 0.5 ml of $NaNO_2$ (1×10^{-3} M) was added, then added 0.5 ml of HCl (2 M), and wait 10 minutes. 1 ml of ADB (500 µg/ml) was added and then added (1 ml) of NH_4OH (1 M) and after 10 min. the volume completed with ethanol, giving the highest absorption of the resulting compound 0.879 at room temperature at 368 nm**.**

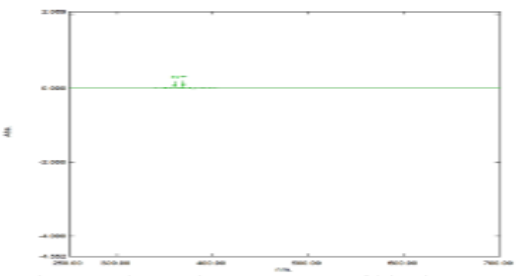


Fig. (9): Absorption spectrum of blank against ethanol

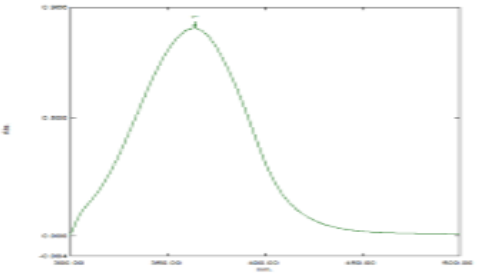


Fig. (10): Absorption spectrum of (ADB) product against blank

"Optimal conditions"
Effect of reagent volume

The reaction between p-nitroaniline with (ADB) was studied at room temperature, when adding varying volumes of p-nitroaniline at an initial concentration of (1×10^{-3} M). It is clear from figure (11), that the best added volume is (1 ml).

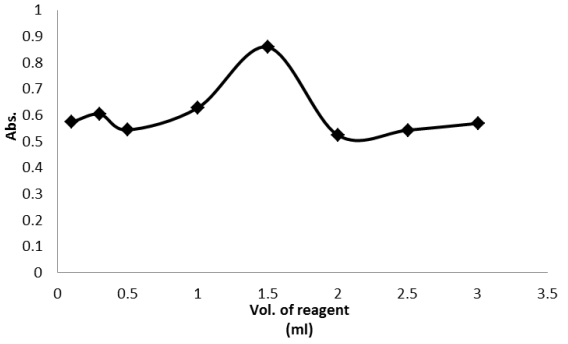


Fig. (11): Effect of reagent volume

Effect of $NaNO_2$ volume

Increasing and different volume of ($NaNO_2$) (1×10^{-3} M) were investigated, the volume that gives the best absorption is 0.5 ml, as shown in figure 12.

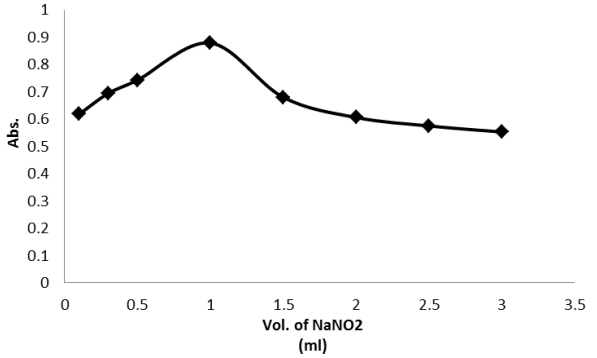


Fig. (12): Effect Vol.(ml) of $NaNO_2$

Effect of acid volume

Different and increasing volumes of Hydrochloric acid at a concentration of (2 M), were studied, the best volume is found to be (0.5 ml), as shown in figure 13.

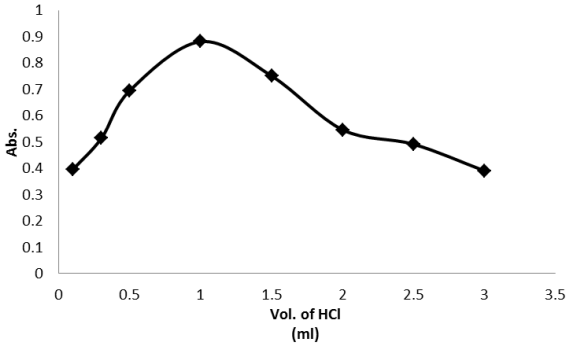


Fig. (13): Effect Vol. (ml) of HCl

Effect of acid type

The acids used are (CH_3COOH, HNO_3, HCl, H_2SO_4) at a concentration of (1.0 M) for each, the same volume added (0.5 ml) to find the acid that gives the best absorption, table (5) shows that the best acid was HCl.

Table (5)
Effect of different types of acids on the product formation

Acid	Absorbance
H_2SO_4	0.510
HNO_3	0.472
HCl	0.875
CH_3COOH	0.603

Effect of the base type

Bases at a concentration of (1 M) were used for each of the bases (NH_4OH, NaOH, KOH), where (1ml) was added to find the base that gives the highest absorption. The best absorption was obtained when using NH_4OH base (table 6).

Table (6)
Effect of different types of bases on the product

Base	Absorbance
NH_4OH	0.874
NaOH	0.399
KOH	0.490

Effect of base volume

Increasing volume of (NH_4OH) base at a concentration of (1 M) were used to find the volume that gives the highest absorption, the best volume is (1 ml), as shown in figure 14.

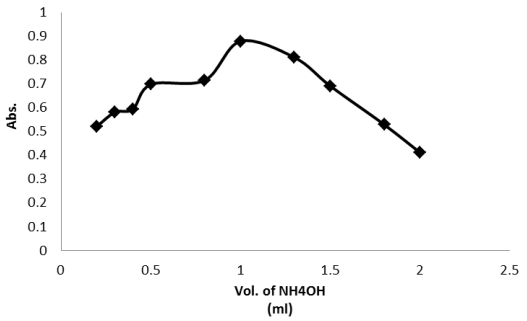


Fig. (14): Effect of NH_4OH volume

Effect of addition sequence

A number of tests were conducted to study the effect of changing the sequence of adding reactants on the absorption of the product, it was found that the addition sequence of number 4 have highest absorption of the product, as in table (7).

Table (7)
Effect of addition sequence

Order number	Order of addition	Absorbance
1	D + R + A + O + B	0.460
2	R+ O + D + A + B	0.680
3	R + A + O + D + B	0.391
4	R + O + A + D + B	0.877
5	D + R + O + A + B	0.374
6	D + A + R + O + B	0.573
7	R + D + O + A + B	0.472

ADB = D, p-nitroanilin = R, HCl acid = A, NH_4OH = B, $NaNO_2$ = O

Effect of time on product stability

The effect of stability of the product was studied for its importance in knowing the period of time in which it remains constant, the interaction with time was followed using optimum conditions every 5 minutes. The product is stable for more than one hour (Table 8).

Table (8)
Effect of time on stability of product

Time (min.)	Absorbance
0.0	0.591
5	0.798
10	0.869
15	0.871
20	0.870
25	0.871
30	0.872
35	0.871
40	0.871
45	0.870
50	0.869
55	0.866
60	0.865
65	0.859
70	0.855
75	0.851

Effect of Additives

Table (9) shows the effect of additives, on the RE value no effect was observed on absorption values.

Table (9)
Effect of additives

Interference	Added con. µg/ml	% RE	Added con. µg/ml	% RE
Cellulose	100	-3.07	200	-2.04
Mognisum stearate	100	0.45	200	1.47
Sodium carbonate	100	-3.86	200	-0.34
Calcium carbonate	100	-2.61	200	-1.36
Mognisum arbonate	100	0.34	200	-2.50

Calibration curve

The calibration curve showed a linearity at concentration rang of (10-200) µg/ml for (ADB) pure form with p-nitroaniline, as shown in figure (15).

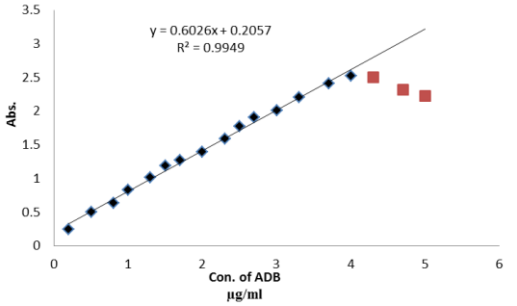


Fig.(15): Calibration curve of (ADB- p-nitroaniline) product

The characteristies of ealibration curve are shown in (table 10)

Table (10)
Characteristics of the calibration curve for spectrophotometric determination of (ADB) product

Parmeters	ESO
$\lambda_{max.}$ (nm)	368
Beer's law (µg\ml)	(10 – 200)
Molar absorptivity (L\mol.cm)	3.42×10^5
Correlation coefficient (r)	0.9974
Limit of Detection (µg\ml)	0.01
Slope	0.6026
Intercept	0.2057
%RSD	0.62

Application of the proposed method

In table (11), the results for determination of (ADB) in the pharmaceutical preparations (as tablets).

Table (11)
Determination of (ADB) (as tablet)

Pharmaceutical preparation	Content (µg/ml) declared	Found (µg/ml) by proposed method	%Recovery
Amlodipine (Amlong) Micro	25	25.24	100.96
	40	40.19	100.48
	50	49.85	99.7
Amlodipine (Accord) UK	25	25.06	100.24
	40	39.79	99.48
	50	50.67	101.34

Equation of the resulting product

Under optimal conditions, the "stoichiometric" of the reaction, was studied with by mole ratio (at initial concentration 1×10^{-3} M), and continuous varations (at initial concentration 1×10^{-3} M) methods. The equivalence between the reagent and this drug was 1:1 (Figures 16 ,17).

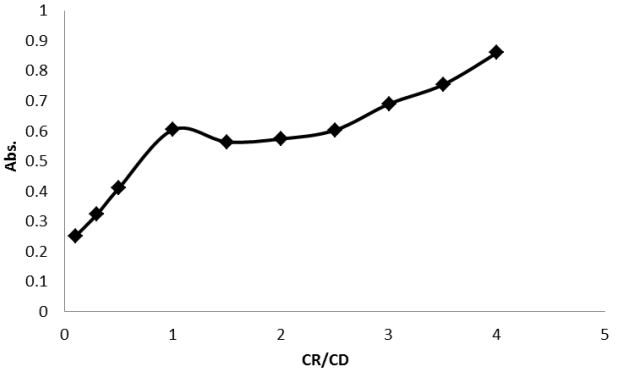


Fig. (16): Mole-ratio method of ADB

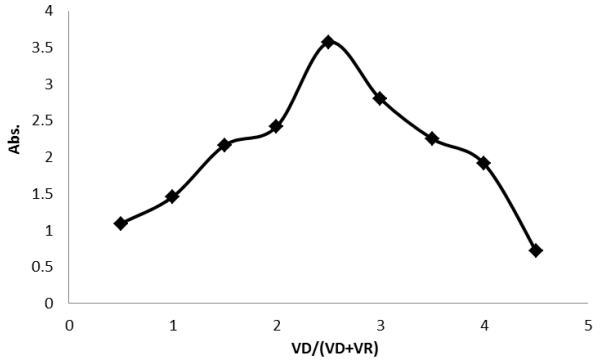
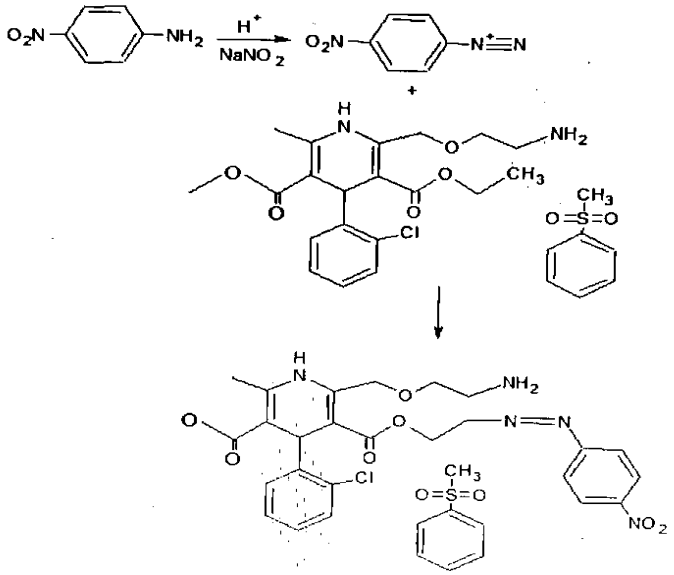


Fig.(17): Continuous variation method of ADB

Suggested interaction

The proposed pathway of the reaction of the developed method may be occurred as following the reaction equation[19,20]:



Conclusion

These methods described here are rapid, simple and do not requires special working conditions unlike many other reported methods. The procedures showed shorter reaction time, stable colored species with inexpensive reagents. The determination can be product performed at room temperature and do not require heating step. The proposed methods can be applied for the determination of ADB in pharmaceutical preparation (Tablet).

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