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Determination of Amlodipine Besylate in its pharmaceutical preparations using spectrophotometric methods

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ABSTRACT— Two simple study in this research sensitive, rapid and unexpansive are schiff's base and oxidative coupling reaction for determination of Amlodipine Besylate (AML) ether in pure form or in it's tablets. The methods were: (I) Synthesis of a Schiff's base (in basic medium) between (AML) and Benzaldehyde, this form a yellow product which has the highest absorption at λ_{max} (383 nm). (II) Oxidative coupling reaction between (AML) and Phenylhydrazine in basic medium to form yellow product of absorbace at λ_{max} (364 nm). Beer's law is obeyed from (10-165) µg/ml with a molar absorptivity (1.91×10^5) L/mole.cm. and the limit of detection is (0.02) µg/ml for the first method and (5-150) µg/ml with a molar absorptivity (1.59×10^5) L/mole.cm. and the limit of detection is (0.02) µg/ml for the second method. In this proposed methods were successfully applied to the determination of (AML) in pure form and in pharmaceutical preprations.

KEYWORDS: Oxidative coupling, Spectrophotometry, Amlodipine besylate, Benzyldehyde, phenylhydrazine.

1. INTRODUCTION

Amlodipine Besylate (AML) belongs to the dihydropyridine family. It is calcium channel blocker with the chemical name (4R,S)-3-ethyl-5-methyl-2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methy pyridine-3,5-dicarboxylate monobenzene sulphonate [1], [2].

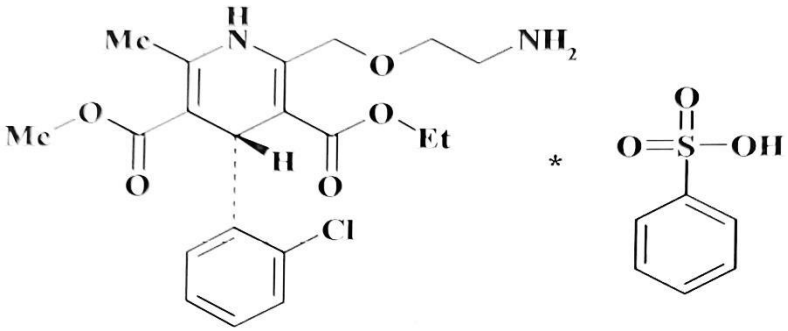

Fig. 1: Chemical structure of (AML)

(AML) use as drugs for treat cardioras cular diseases [3], [4] (AML) is lowering the cytosolic free calcium concentration by blocking calcium pass into cells by blocking calcium cell receptor thus, (AML) has a clear effect on the relaxing and contracting smooth muscles in the body [5]. On that basis, these drugs act as a blood pressure regulator [6]. Analytically, applications of describes the reversed phase high performance

liquid chromatographic method [7] for determination of the drug in bulk and pharmaceutical formulation. Other methods based on high performance liquid chromatography [8- 13], high performance thin layer chromatography [14- 17], gas chromatography [18], gas chromatography coupled with mass spectrometry [19], liquid cheomatography coupled with tandem mass spectrometry [20], fluorimetry [21] and reversed phase high performance liquid chromatography [22- 24] have been described in the literature.

2. The research aims

In the present study an attempt has been made to develop two spectrophotometric methods for the quantitative estimation of (AML). The first converting it to its reduced form by using benzyldehyde in basic medium, the second oxidative coupling reaction by phenylhydrazine in basice medium, That two methods are sensitive, rapid, simple and successfully applied to determine of (AML) in their pharmaceutical formulations.

2.1 Apparatus

UV-Vis spectrophotometer double beam (shimadzu UV Spectrophotometer (UV-1800)) with 1 cm quartz cell's, oven (Memmert, Schutzart DIN 40050-Ip20), balance (KERN ABS 120-4N).

2.2 Materials

Amlodipine Besylate 99% from (SDI Samarra-Iraq). Phenylhydrazin 99% from (SDI Samarra-Iraq), KIO_3 98% from (Merck), NaOH 98% from (Fluka), Benzyldehyde 99% from (Scharlau).

2.3 Solutions

- Amlodipine Besylate (1000 µg/ml): dissolve (0.1 gm) of (AMB) in (100 ml) of absolute ethanol.
- Benzaldehyde (2 M): dissolved (10.293 ml) in (50 ml) of distilled ethanol.

-Sodium hydroxide (2 M): prepared by dissolving (8 gm) in (100 ml) of distilled water.
(1 M): prepared by dissolving (4 gm) in (100 ml) of distilled water.

-Phenylhedrazin (0.2 M): (2.1628 gm) dissolved in (100 ml) of distilled water.

-KIO3 (1×10-2 M): (0.2140 gm) dissolved in (100 ml) of distilled water.

3. General procedures

3.1 (I) Determination of (AML) by Benzaldehyde as reagent

After several initial experiments the optimum conditions were reached where transferred (1.0 ml) of (AML) solution (500 µg/ml) to 10 ml volumetric flask, then (4 ml) of benzaldehyde at concentration (2 M) were added to it, then (1 ml) of the NaOH base (2 M), after of 10 min. Supplemented the volume with ethanol, and the highest absorption of the product was 0.718 at room temperature at 383.5 nm.

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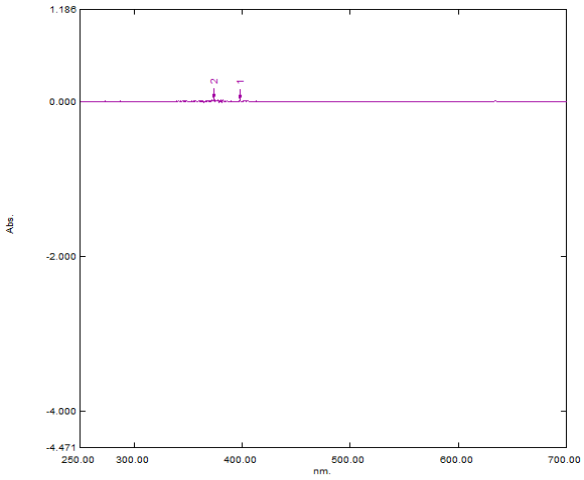

Fig. (2): Absorption spectrum of blank against ethanol

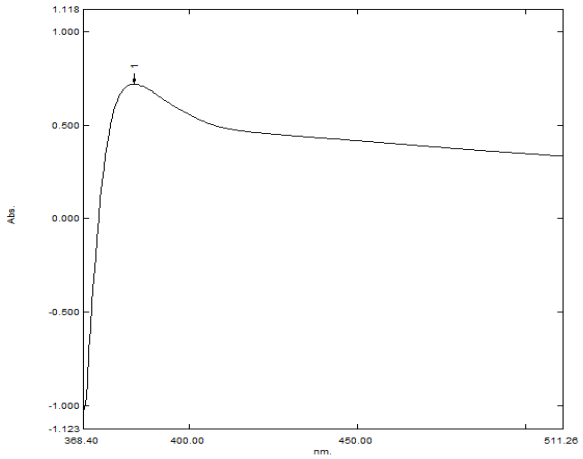

Fig. (3): Absorption spectrum of (AML) product against blank

3.2 Application of proposed methods

Ten tablets of (AMB) were weighted and powdered after, dissolved in least amount of ethanol and then filtered, washed with ethanol solvent and the washws were collected in a volumetric flask (50 ml). The final concentration of the resulting solution was (500 µg/ml) it was successful suggested methods of (AMB) in various commercial tablets.

4. Results and Discussion

4.1 "Optimal Conditions"

4.1.1 Effect of reagent volume

Added increased volumes of the reagent (Benzyldehyde) with an initial concentration (2 M), In order to see how effective the absorotion values of the product, as shown in the figure (4), It is found that the best absorption was at a volume of (4 ml).

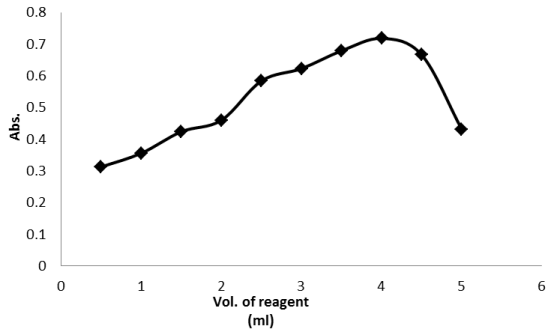

Fig. (4): Effect of Benzaldehyde volume on product

4.1.2 Effect of base volume

Increasing volumes of NaOH solution have been added with a concentration of (2 M), It was found that the best added volumes are (1 ml), as shown in fig. (5).

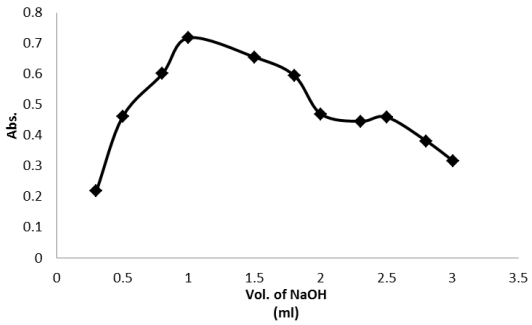

Fig. (5): Effect of NaOH volume on the product

4.1.3 Effect of different bases

Using several base solutions, including (NaOH, KOH, NH_4OH) and they were all at a concentration of (2 M) and adding the same volume of (1 ml), the best absorption was obtained when using NaOH, as shown in table (1).

Table (1): Effect of different bases on the product

Base	Absorbance
KOH	0.600
NaOH	0.715
NH_4OH	0.423

4.1.4 Effect of time

The stability of the product is very important to knowing the period of time which the product remains constant, the stability of the absorption values was observed at approximately 60 minute, (1 hour), and this is suffrcient time to make the required measurements, table (2).

Table (2): Effect of time on stability of product

Time (min.)	**Absorbance**
5	0.697
10	0.702
15	0.709

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20	0.713
25	0.715
30	0.717
35	0.718
40	0.718
45	0.718
50	0.716
55	0.710
60	0.712
65	0.707
70	0.701
75	0.695
80	0.652

4.1.5 Effect of additives

The effect of additives on the composition of the product was studied, and not observed any effect, as shown in the table (3).

Table (3): Effect of additives

Additives	Added con. µg/ml	%RE	Added con. µg/ml	%RE
Cellulose	100	0.98	200	-1.53
Magnesium stearate	100	0.28	200	-0.70
Sodium carbonate	100	-2.79	200	2.23
Calcium carbonate	100	-1.95	200	2.09
Magnesium carbonate	100	1.81	200	-1.11

4.1.6 Calibration curve

Figure (6) showes a linearity at concentrations rang of (10-165) µg/ml.

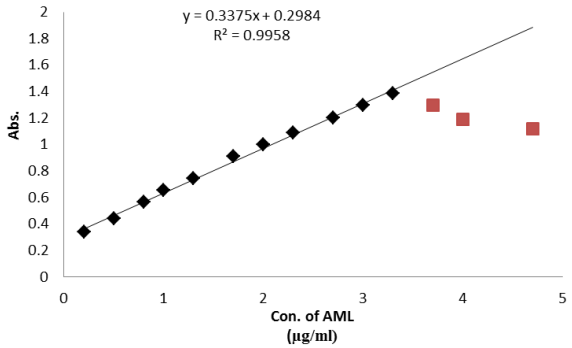

Fig. (6): Calibration curve of (AML- Benzaldehyde) product

The characteristies of ealibration curve are shown in (table 4)

Table (4): Characteristics of the calibration curve for spectrophotometric determination of (AML) product

Parameters	**AML**
λ_{max}. (nm)	383
Beer's law (µg\ml)	(10 – 165)
Molar absorptivity (L\mol.cm)	1.91×10^5

Correlation coefficient (r)	0.9979
Limit of Detection (µg\ml)	0.02
Slope	0.3375
Intercept	0.2984
%RSD	0.61

4.1.7 The stoichiometry of the product

The "equivalence of the product" was studied for the interaction of (AML) with the reagent under optimal conditions by the molar ratio method, as the initial concentration used was 1×10^{-2}, as well as by the continuous change method at an initial concentration of 1×10^{-2}, where the ratio of equivalence between the drug and the reagent was (1:1), (Figures.7,8)

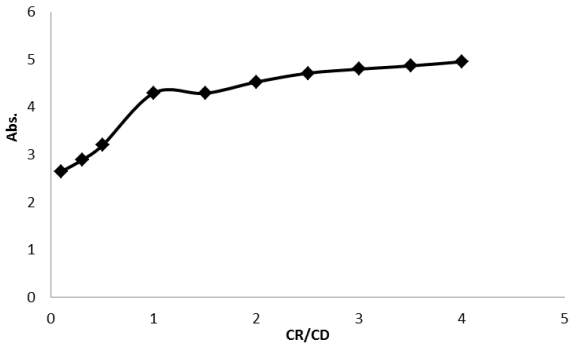

Fig. (7): Mole-ratio method of AML

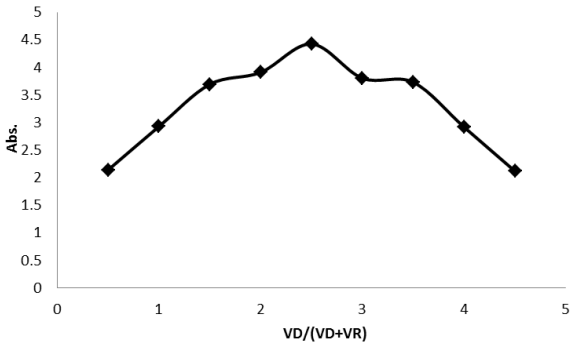

Fig. (8): Continuous variations method of AML

4.1.8 Application of the "proposed method"

In table (5), the results of determination of (AML) in the pharmaceutical preparation (as tablets).

Table (5): Determination of (AML) in commercial tablets

Pharmaceutical preparation	Content (µg/ml) declared	Found (µg/ml) by proposed method	%Recovery
Amlodipine (Amlong) Micro	40	40.58	101.45
	85	85.07	100.08
	115	114.96	99.97
Amlodipine (Accord) UK	40	39.55	98.88
	85	85.02	100.02
	115	115.39	100.34

4.1.9 Suggested interactions

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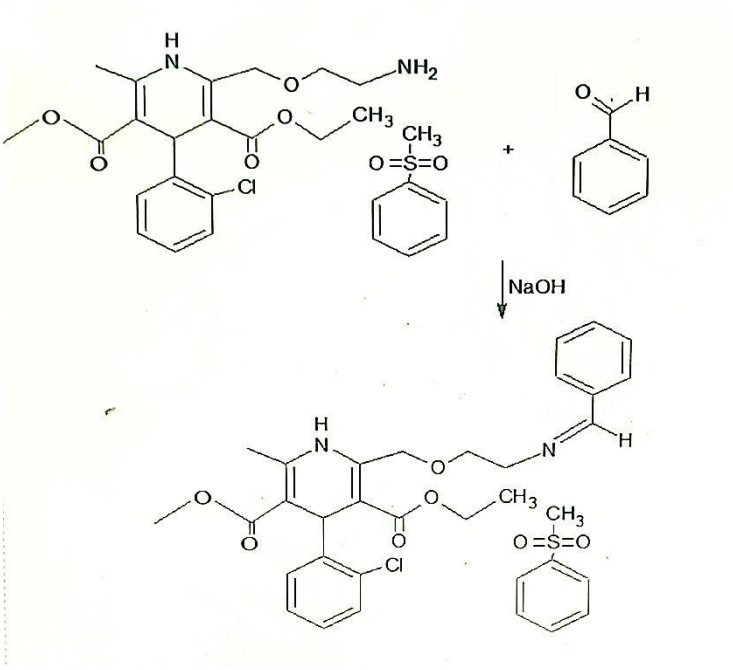
The proposed reaction can be based on condensation reaction between AML and Benzyldehyde in a base medium to produce a dense yallow colored product, (Schiff's base) [25- 27].



5. (II) Determination of reaction between (AML) and phenylhydrazine as reagent by oxidative coupling reaction

To a volumetric flask of 10 ml, transferred 1 ml of (AML) at the concentration of (250 µg/ml) to it, then (1 ml) of oxidizing agent (KIO_3) at the concentration of (1×10^{-2} M) was added, then (0.5 ml) added from NaOH at the concentration of (1 M), after waiting for 15 min. (1 ml) of phenylhydrazine was added then the volume is completed to (10 ml) with absolute ethanol and the maximum absorption of the resulting product (yallow color) was 0.361 and λ_{max} was 364 nm at room temperature.

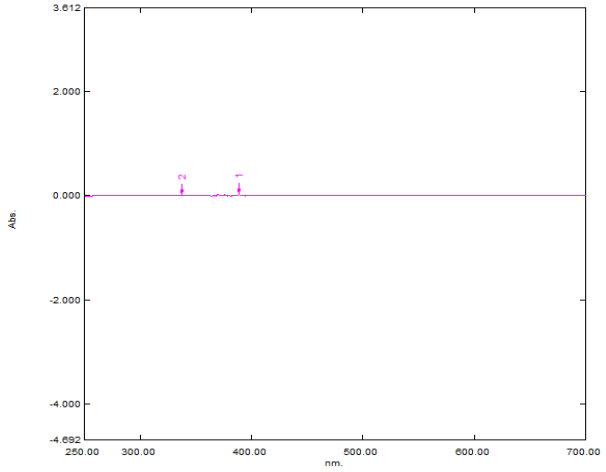


Fig. (9): Absorption spectrum of blank against ethanol

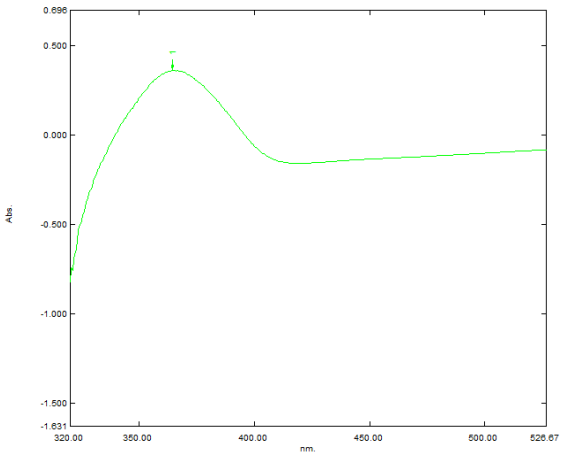


Fig. (10): Absorption spectrum of (AML) product against blank

5.1 "Optimal conditions"

5.1.1 Effect of reagent volume

Figure (11), shows the effect of adding increasing volumes of phenylhydrazine on the absorption of the product, that best added volume was (1 ml).

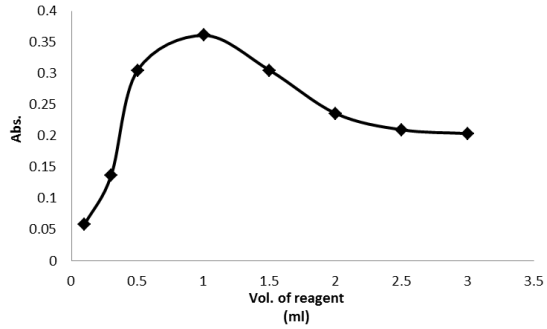


Fig. (11): Effect of phenylhydrazine volumes on product

5.1.2 Effect of oxidation agent volume

It is clear from Figure (12) that the best added volume of the oxidizing agent is 1.0 ml which was used in subsequent studies.

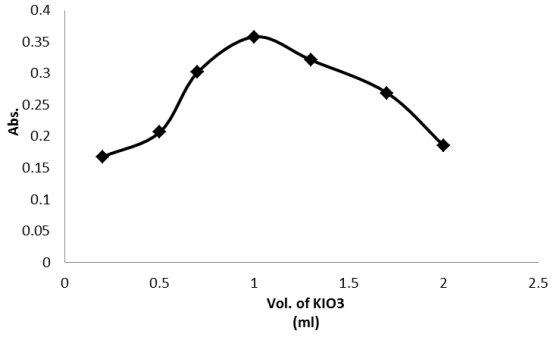


Fig. (12): Effect of oxidative agent volume on product

5.1.3 Effect of base volume

Different and increasing volumes of NaOH were used at a concentration of (1 M), were studied, as shown

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in figure (13), the optimal added base volume is 0.5 ml.

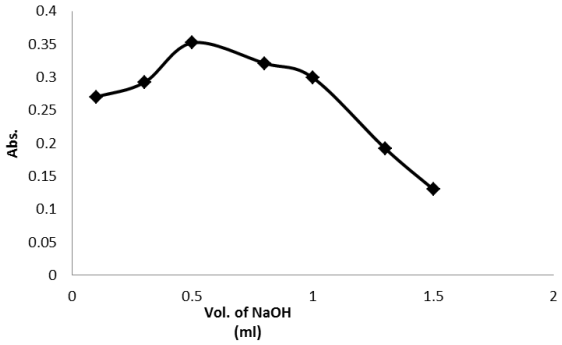


Fig. (13): Effect of NaOH volumes on product

5.1.4 Effect of base type

The bases were used (NaOH, KOH, NH_4OH) with the concentration of (1.0 M) for each, as well as the same volume added (0.5 ml), to know wich base gives the best absorption, when product formation, table (6) shows that the best base used to form the product is NaOH.

Table (6): Effect of different types of bases on absorption values of the (AML) product

The base used	Absorbance
NaOH	0.358
KOH	0.247
NH_4OH	0.254

5.1.5 Effect of time on stability of product

(Table 7) shows the stability of the absorption product values at λmax 364 nm with time, the absorption value of the product is fixed for 60 minutes, (1 hour).

Table (7): Effect of time on stability of product

Time (min.)	**Absorbance**
5	0.321
10	0.332
15	0.344
20	0.351
25	0.359
30	0.360
35	0.360
40	0.361
45	0.363
50	0.360
55	0.358
60	0.355
65	0.341
70	0.337
75	0.331
80	0.326

5.1.6 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 have highest absorption of the product, as in table (8).

Table (8) Effect of addition sequence

Order number	Order of addition	Absorbance
1	D + O + B + R	0.354
2	D+ R + O + B	0.246
3	D + O + R + B	0.279
4	R + O + B + D	0.055

AML = D, Phenylhydrazine = R, NaOH base = B, KIO_3 = O

5.1.7 Effect of additives

The effect of additives the composition of the product was studied, and there are no effect of additives on absorption values, as shown in table (9).

Table (9): Effect of additives

Additives	Added con. µg/ml	%RE	Added con. µg/ml	%RE
Cellulose	50	-1.88	100	1.72
Magnesium stearate	50	2.22	100	-1.35
Sodium carbonate	50	-1.19	100	-2.58
Calcium carbonate	50	3.92	100	-4.27
Magnesium carbonate	50	-1.36	100	-3.24

5.1.8 Calibration curve

Figure (14) shows the linearity at concentration rang of (5-150) µg/ml.

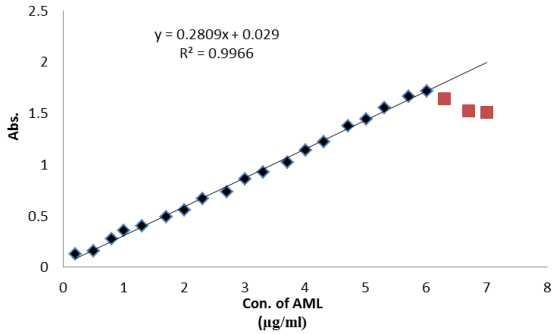


Fig. (14): calibration curve of (AML) product

5.1.9 Application of the proposed method

The result for determination of (AML) in the pharmaceutical preparations (as tablets).

Table (10): Determination of (AMB) (as tablet)

Pharmaceutical preparation	Content (µg/ml) declared	Found (µg/ml) by proposed method	%Recovery
Amlodipine (Amlong) Micro	20	19.88	99.4
	50	50.31	100.62
	75	74.69	99.59
Amlodipine	20	20.17	100.85

(Accord) UK	50	49.05	98.10
	75	74.24	98.99

5.1.10 Construction of calibration curve

The characteristies of ealibration curve are shown in (table11)

Table (11): Optical characteristics of the calibration curve for spectrophotometric determination of (AML) product

Parameters	AML
λ_{max}. (nm)	364
Beer's law (µg\ml)	(5 – 150)
Molar absorptivity (L\mol.cm)	1.59×10^5
Correlation coefficient (r)	0.9983
Limit of Detection (µg\ml)	0.02
Slope	0.2809
Intercept	0.029
%RSD	1.04

5.1.11 The stoichiometry of the product

The "equivalence of the product" was studied for the interaction of AML with the reagent phenylhydrazine under optimal conditions by the molar ratio method, as the initial concentration used was 2×10^{-3}, as well as by the continuous change method at an initial concentration of 2×10^{-3}, where the ratio of equivalence between AML and the reagent was 1:1, as shown in Figures. 15, 16.

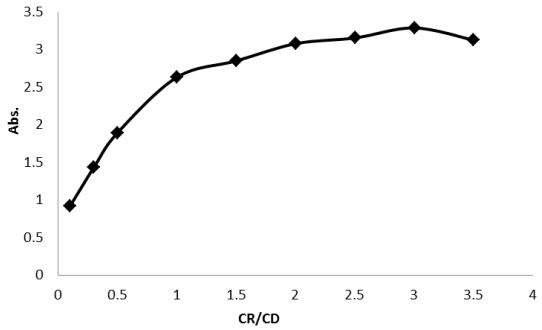


Fig. (15): Mole-ratio method of AML

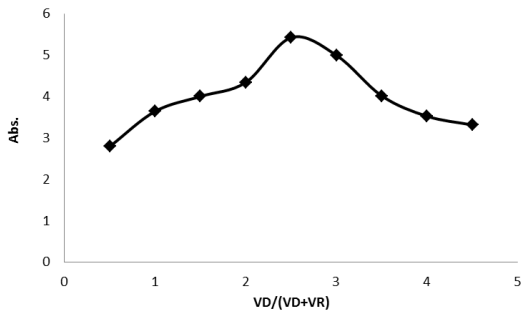
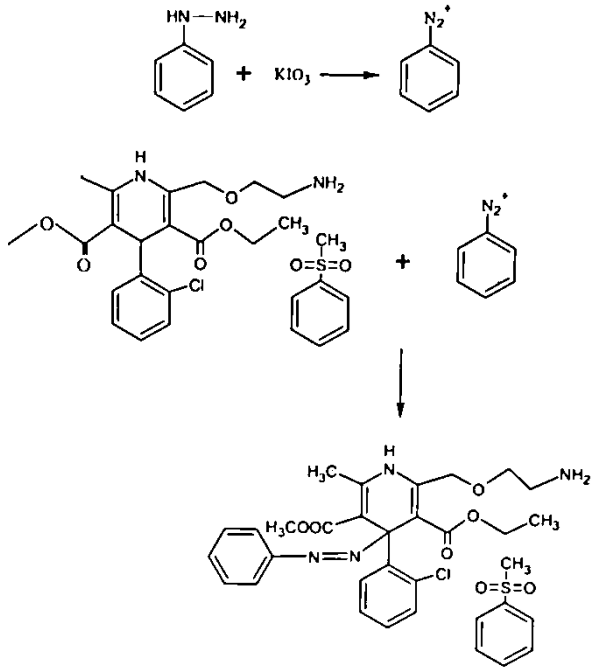


Fig. (16): Continuous variation method of AML

5.1.12 Suggested interactions

The proposed reaction can be based on how the AML drug is oxidized and coupling reaction [28], [29].



6. Conclusions

These proposed methods are simple and fast and do not require complex working conditions and showed a quick response compared to other methods, as they gave color results with reagents that are inexpensive financially. Thus, these two methods can be applied for the determine AMB in its pharmaceutical preparations.

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