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Developments of HPLC Method for Determination of Methyl Dopa

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ABSTRACT

This research contains the development of accurate and quick method for determination methyldopa drug using high performance liquid chromatographic technique, injected 20 µl of the drug solution with concentration 20 µg/ml using a column type C18 , mobile phase consists of Acetonitrile: 0.02M KH2PO4 (70 : 30) at flow rate of 1.6 m1/min using UV detector at 275nm, pH5.65 ,with retention time 2.89min at linear range 10 – 50µg/ml with good accuracy and precision . The recovery value is to 99.2%and detection limit of 0.05 µg/ ml. The method is applied successfully to determine methyl dopa in its pharmaceutical formulations.
Keywords: Chromatography, Methy Dopa, Acetonitrile, Drug

الملخص

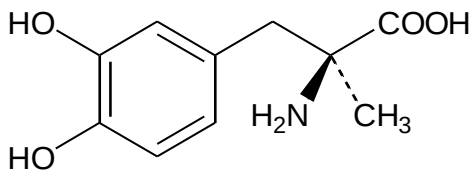
تضمن هذا البحث تطوير طريقة دقيقة وسريعة لتقدير المثيل دوبا بأستخدام تقنية كروماتوغرافيا السائل عالي الاداء اذ تم حقن 20 مايكروليتر من محلول العقار بتركيز 20 مايكروغرام / مل بأستخدام عمود من نوع C_{18}: وطور متحرك يتكون من اسيتونتريل 0.02 مولاري (70 : 3) KH_2PO_4 البنفسجية عند الطول الموجي275 نانوميتر عند الدالة الحامضية 5.56 ، وزمن احتجاز مل / دقيقة ومكشاف الاشعةفوق1.6وبمعدل سرعة جريان قيمةالاسترجاعية99.2 % بلغت جيدين اذ وضبط دقة 2.89 دقيقة وكان مدى خطية التراكيز 10 - 50 مايكروغرام / مل وذات مستحضراتهاالصيدلانية. لتقديرالمثيل دوبا في بنجاح الطريقة وتطبيق وحدالكشف0.05مايكروغرام /مل

الكلمات الافتتاحيه: كروموتوغرافيا ,الطور المتحرك , الطور الثابت

I. INTRODUCTION

Scientific names of methyldopa

L-alpha-methyl-3,4-dihydroxyphenyl alanine
L-2-amino-2-methyl-3-(3,4-dihydr oxy phenyl)propionic acid
2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid composition formula Its



Molecular formula**:** $C_{10}H_{13}NO_4$, 1½ H_2O, MW: 238.2 g/mol**,** uv max : 281nm

A white or yellowish white, crystalline powder or colourless or almost colourless crystals, slightly soluble in water, very slightly soluble in alcohol, practically insoluble in ether. It is freely soluble in dilute mineral acids, practically insoluble in chloroform and ether. M.p. about 310°C[(1)]. It is used as antihypertensive and it exists as Tablets. It has the following commercial names Aldomet, Aldoril, Dopamet, Dopegyt, Aldosam, Aldopren , Amender, Cardin, Dopegyt, Hipten, Hydopa, Isomet, Medimet-250 , Medomet, Medopren, Meldopa, MetalphaNova- Medopa, Nudopa, Nu-Medppa, Presinol, Prodop, Pulsoton , Selm, Sembrina, Tenzone[(2)].

II. METHODS AND MATERIAL

Methods of estimating Methyl dopa : Chromatographic methods[(3 -9)] Spectroscopic methods [(11)] Experimental Works Apparatus

1-Apparatus of High Performance Liquid chromatography of type schematize UV / VIS – HPLC Series 200 JAPAN mode and supplied with Column of Type L1 – macherey – NOGEL (MN) – (25CM X 4.6 mm . 5MM) Porous silica Particles 5 to 10 Micro min diameter**,** Japan.

2-Shimadzu UV-160, UV-Visible computerized double-beam spectroph-otometer , Japan.

3- The pH measurements are carried out using Philips PW 9421 pH meter

Reagent and Chemicals

Chemical material and analytical reagents of high purity were used and as in the Table (1)

Table 1 : Reagent and ChemicalsTable (1)

Chemicals	**Chemical Structure**	**The percent Purityof**	**Company**
Methyldopa	$C_{10}H_{13}NO_4$,1½H2O	99.8%	SDI\ Iraq
Potassium di hydrogen phosphate	KH_2PO_4	99%	BDH
Potassium hydroxide	KOH	99.8%	BDH
Acetonitrile	CH_3CN	99%	Fluka
Methanol	CH_3OH	99%	Fluka
Phosphoric acid	H_3PO_4	99%	BDH

Preparation of the Solutions

1-Solution of the stock methyldopa (1000 µg/ml).

This solution is prepared by dissolving 0.1000 g of methyldopa powder in distilled water and the volume is completed to 100 ml with distilled water in volumetric flask.

2-Solution of methyldopa (100 µg/ml)

This solution is prepared by diluting10 ml of the above stock methyl dopasolutionto100 ml in a volumetric flask with distilled water.

3-Solution of methyldopa (20 µg/ml)

This solution is prepared by diluting 5 ml of the above stock methyldopa solution to 25 ml in a volumetric flask with distilled water.

III. RESULTS AND DISCUSSION

1- Selection of column

The best separation column is selected for the chlorpromazine hydrochloride drug of type (porous silica particles 5 to 10 micron) C_{18} at 250 mm length and 4.6 mm diameter due to its high separation efficiency according to the previous research [(10)].

2-Selection of wavelength (λ max)

Asolution of methyldopa (1000 µg/ml) is prepared and measured the absorption by using quartz cell of 1cmwidth.Figure (1) shows that the optimum λ max is at 281 nm. This wavelength is therefore adopted for HPLC experiment.

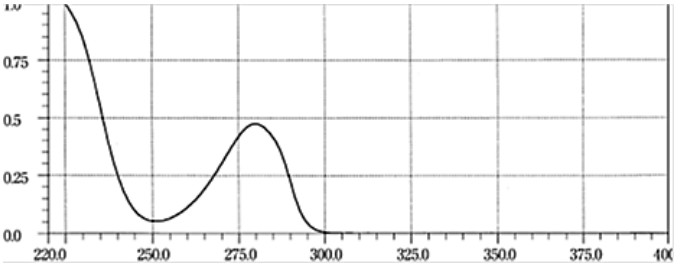


Figure 1 : UV-Visible spectrum of methyldopa solution (1000 µg/ml)

3- Selection of mobile phase

When injection 20 µl of methyldopa solution (20 µg/ml) using various mobile phases at wave length 281nm and flow rate 1ml/min, The results are shown in Table (2) Figure (2).

Table (2) Selection of mobile phase

Mobile Phase	Rt. (min)	Peak Area (mV)	Peak Height (mV)	N	HETP
Acetonitrile: 50 KH_2PO_4 : 30 Methanol : 20	5.152	600934.1	7668.7	107.17	0.2354
Acetonitrile: 50 KH_2PO_4 : 50	4.767	575358.0	7898.6	363.5	0.06
Methanol : 50 KH_2PO_4 : 50	5.648	683685.5	7956.2	Bad Separation	
Acetonitrile: 50 Methanol : 50	5.66	617301.0	6070.7	Bad Separation	
Acetonitrile: 70 KH_2PO_4 : 30	**4.777**	**767533.2**	**8365.8**	**1014.21**	**0.0246**

We can observe from the above Table that the best mobile phase is in Figure (2)because it gave the highest number of theoretical plates (1014.21),low HETP (0.0246) and less retention time (4.777)min , therefore, it will be adopted in the subsequent experiments.

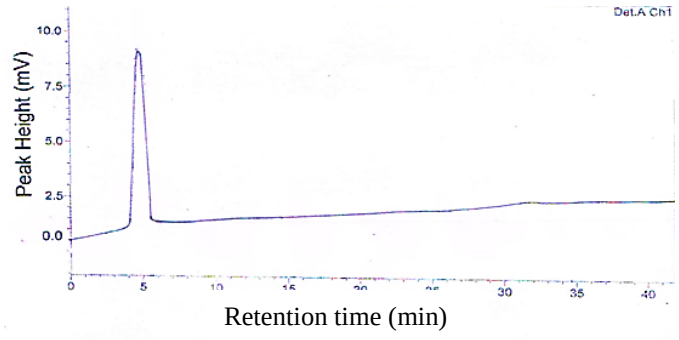


Figure 2 : Chromatogram injection 20 µl of methyldopa solution (20 µg/m l) by mobile phase with ratio of Acetonitrile 70 : KH_2PO_4 30

4- Effect of wavelength

After choosing the (Acetonitrile: KH_2PO_4 with ratio 70: 30, 20) µl of methyldopa solution (20 µg/ml). Signal (peak area) was recorded at different wave lengths (280, 260 , 290 , 285 , 275) nm . The results are shown in Table (3) and Figure (3).

Table (3) Selection of wavelength

λmax nm	Rt. (min)	Peak Area (mV)	Peak Height (mV)	N	HETP
260	Separation is not good				
275	**4.595**	**513538.6**	**62173**	**1351.29**	**0.018**
280	4.5	649874.5	7861.0	900	0.027
285	4.604	616625.6	76504	1356.5	0.018
290	4.591	445767.2	5457.0	Separation is not good	

From the above Table it was found to be 275nm is the best wave length which is showing in Figure (3)because it gave the highest number of theoretical plates (1351.29), low HETP (0.018) and less retention time (4.595)min , therefore , it will be adopted in the subsequent experiments.

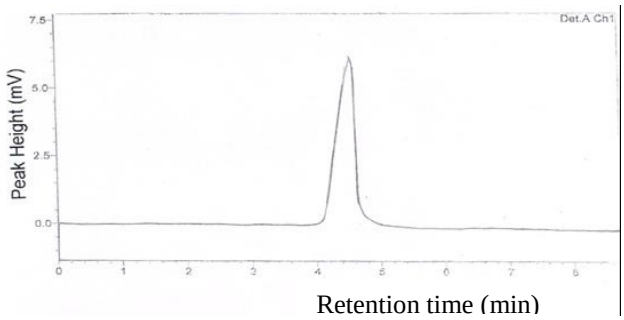


Figure 3: Chromatogram injection 20 µl of methyldopa solution (20 µg/m l) at 275nm

5- Effect of pH

The mobile phases (Acetonitrile : KH_2PO_4 with ratio 70 : 30) at various pH (5.65 , 6.66 , 7.8 ,4.6 , 5) are prepared by using the solutions of phosphoric acid(0.1 M) and potassium hydroxide (0.1 M). The response (peak area) is measured for each mobile phase at various pH, and the results are shown in Table (4) and Figure (4).

Table 4 : Effect of pH

pH	Rt. (min)	Peak Area (mV)	Peak Height (mV)	N	HETP
4.6	4.591	262329.4	3451.9	Separation is not good	
5	4.591	313392.8	3838.2	Separation is not good	
5.65	**4.599**	**290368.9**	**3615.7**	**1353.61**	**0.0184**
6.66	4.607	278351.5	3852.3	530.61	0.0471
7.8	4.555	324764.2	4065.7	409.83	0.0610

From the above Table it was found that the mobile phase of Acetonitrile and buffer solution with ratios (70:30) at pH function 5.65 is choosed due to appearance of a sharp peak is in Figure (4) because it gave the highest number of theoretical plates (1353.61), low HETP (0.0184) and less retention time (4.599) min , therefore , it will be adopted in the subsequent experiments.

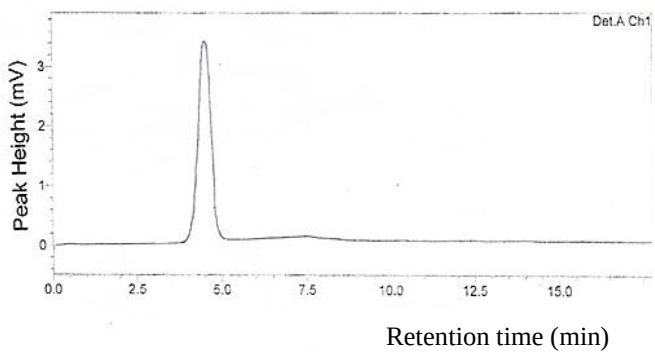


Figure 4 : Effect of pH=5.65 of mobile phase (Acetonitrile 70 : KH_2PO_4 30) on the chromatogram

6-Effect of flow rate

When injection 20 µl of methyldopa solution (20 µg/ml) at flow rate for mobile phase Acetonitrile 70 : KH_2PO_4 30 between (0.8 – 1.8) ml/min. The results are shown in Table (5) and Figure (5).

Table 5 : Effect of flow rate

Flow Rate ml/min	Rt. (min)	Peak Area (mV)	Peak Height (mV)	N	HETP
0.8	5.793	379318.0	3791.6	838.93	0.0291
1	4.599	290368.9	3615.7	1353.6	0.0184
1.2	3.826	310253.5	4140.5	936.84	0.026
1.4	3.296	222845.6	3790.1	1086.36	0.0230
1.6	**2.890**	**196650.3**	**3816.3**	**1484.8**	**0.0168**

We can see from the above Table that the best flow rate is in Figure (5)because it gave the highest number of theoretical plates (1484.8), low HETP (0.0168) and less retention time (2.890)min, therefore ,it will be adopted in the subsequent experiments.

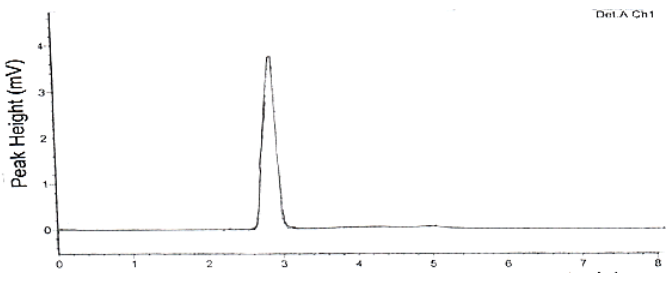


Figure 5: Chromatogram injection 20 µl of methyl dopa solution (20 µg/ml) at flow rate (1.6ml /min) .

Calibration curve-7

After obtaining the optimum conditions 20 µl of different concentrations methyldopa solution a (10 , 20 , 30, 40, 50) µg/ml which prepared from the solution of 1000 µg/ml was injected in column of type C_{18} at wave length 275nm , mobile phase Acetonitrile 70 : KH_2PO_4 30 , pH function 5.56 and flow rate 1.6 ml/ min .The results are shown in Table (6) and Figures (6) .

Table (6) Calibration curve

Conc. Present µg/ml	Rt. (min)	Conc. measured µg/ml	Recovery%	N	HETP
10	2.897	10.08	100.8	1492.0	0.0167
20	2.900	20.7	103.5	1495.1	0.0167
30	2.890	29.64	99.98	1484.8	0.0168
40	2.882	39.78	99.45	1476.6	0.0169
50	2.896	51.00	102.0	1490.9	0.0167

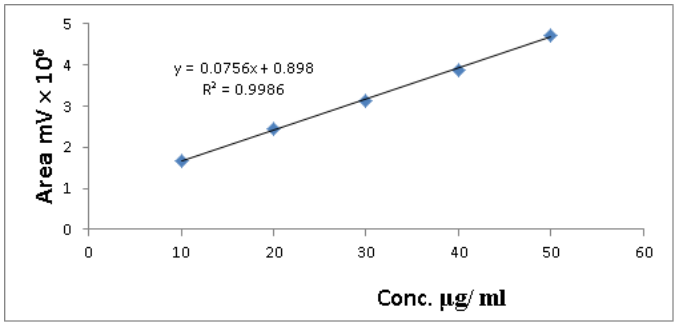


Figure 6: Calibration curve for the determination of methyl

We can see from the above results calibration curve was linear over the concentration range of 10 – 50 µg/ml for methyldopa. The method was accurate, specific and rapid.

8 - Detection limit Detection limit was calculated by measuring the peak area of five injections for the lowest concentration (10 µg/ ml) in the calibration curve and within the limit of the Beer law at the same conditions (optimum conditions), the detection limit was 3.6×10^{-4}µg/ ml, and results are recorded in Table (7).

Table (7) Detection limit

Concentration µg/ ml	$\overline{X}$	S	D.L µg/ ml
10	1656.77	0.02	3.6×10^{-4}

Applications 9

The proposed method has been applied on the preparation pharmacist Aldosam (Samarra/ Iraq) in tablets . Each tablet contains 250 mg methyldopa , has been weight of 10 tablets each tablet alone and crushed well and then calculated the required weight to prepare a solution with concentration of 1000 µg/ml , dissolved in amount of distilled water and complete the volume to the mark in volumetric flask of 100ml. It was prepared and then the solution of 20 µg/ml was prepared and 20µl of this solution is injected in HPLC instruments. The results are shown in Table (8) and (9) and Figures (7) and (8).

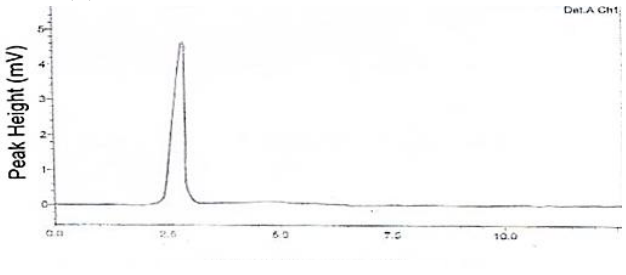


Figure 7: Chromatograms of injection 20 µl of 20 µg/ml of standard methyl dopa

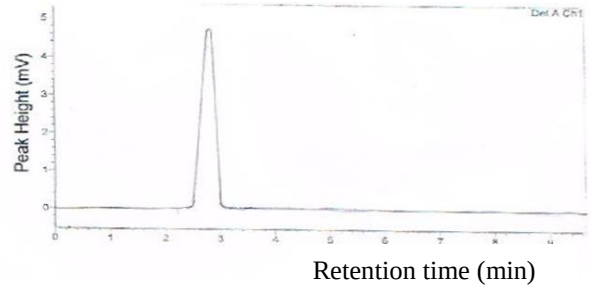


Figure 8: Chromatograms of injection 20 µl of 20 µg/ml of preparation pharmacist Aldosam

Table (8) Results for the determination of methyldopa in standard and pharmaceutical preparation

Sample	Conc. µg/ml	Rt.(min)	*Peak Area (mV)	*Peak Height (mV)	N	HETP
Standard	20	2.900	245604.9	45878.5	1493.11	0.0167
Aldosam Tablets	20	2.896	243760.7	43977.2	1490.90	0.0167

Average of three determinations*

Table (9) Precision and accuracy of the determination of methyldopa in its Aldosam formulation

Conc. Of Aldosam µg/ml			RSD%*	*RE% CC	*Rec.% CC	RE%* SCM	*Rec.% SCM
Present	Measured 0f calibration curve	Measured SCM	0.0503	3.5	103.5	- 0.8	99.2
20	20.7	19.84					

Average of three determinations*

From the above Tables we can see that the application of the proposed method on the preparation pharmacist Aldosam gives good results, the value of RSD 0.0503% and high recovery 99.2%

Comparison with other methods 10

The proposed method was compared with other methods and the results are shown in Table (10).

Table (10) Comparison with other methods

Analytical Parameter	Literature[19] method	Present Method
Mobile phase	Acetonitrile: distilled water: triethylamine (45:45 :10)	Acetonitrile : KH_2PO_4: 70:30
Column	C_{18}	C_{18}
Flow rate ml/min	1.5	1.6
Retention time (min)	6.499	2.89
λ_{max} (nm)	245	275
pH	3.0	5.56
Recovery (%)	102.9	99.2
RSD (%)	0.0493	0.0503
Coefficient of correlation	0.998	0.998
Type of HPLC	RP-HPLC	RP-HPLC
D.L µg/ ml	0.029	3.6×10^{-4}
Pharmaceutical preparation	Tablet	Tablet

The results of the above Table indicate that the method used in the present work is sensitive and economical because it needed low quantity of mobile phase and low retention time.

IV. CONCLUSION

A rapid and easy chromatographic method was developed to separate and determined methyldopa drug by using the reversed phase. This method gives an efficiency in good separation by using mobile phase Acetonitrile 70: KH2PO4 30) in column of type C18at pH 5.56 , wavelength 275nm and flow rate 1.6 ml/ min and detection limit attained to 3.6×10- 4µg/ml .The method is applied successfully on determine methyldopa in its pharmaceutical appliances.

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