Spectrophotometric determination of Levo-dopa in pharmaceutical preparation via oxidative coupling organic reaction

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Kay ward : Levo-dopa drug , Spectrophoto Metric determination , Pharmacetical preparation

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Abstract :

A simple, accurate and sensitive colorimetric method for the determination of L-Dopa in pure and pharmaceutical preparations has been established .The proposed method uses 4–aminoantipayrine (4-AAP) as a chromogenic reagent . The method is based on the oxidative coupling reaction of L-Dopa with 4-AAP in the presence of sodium hydroxide as alkaline media to form a red water soluble dye product , that has a maximum absorption at 519 nm . Linearity was observed in the range of (0.20 – 30.00) µg.ml^{-1}, with molar absorptivity of (0.58×10^{4} L.mol^{-1}.cm^{-1}) ,a sandall sensitivity of (0.36×10^{-4}µg.cm^{-2}) , correlation coefficient of 0.9999 , detection limit (0.12 µg.ml^{-1}) and the relative standard deviation is better than (1.013%) . The method was applied successfully for the determination of L-Dopa in pharmaceutical preparations and the value of recovery % was found (99.68 %).

التقدير الطيفي لعقار الليفو دوبا في المستحضرات الصيدلانية بواسطة تفاعلات الاقتران التاكسدي

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مفتاح البحث : عقار الليفو دوبا ’ التقدير الطيفي ، المستحضرات الصيدلانية

الخلاصة :

تم تطوير طريقة لونية بسيطة ومضبوطة وحساسة لتقدير الليفو دوبا في صيغته النقية وفي مستحضراته الصيدلانية . الطريقة المقترحة تعتمد على ازدواج الليفو دوبا مع الكاشف اللوني 4- امينو انتي بايرين وبوجود هيدروكسيد الصوديوم حيث يتكون ناتج احمر يعطي امتصاص اعظم عند الطول الموجي (519)نانو ميتر . اظهرت النتائج ان مدى الخطية بين (0.20-30.00) مكغم .مل$^{-1}$ و بمعامل امتصاص مولاري مقداره (0.58×10^{4}) لتر .مول$^{-1}$.سم$^{-1}$ ودلالة ساندل مقدارها (0.36×10^{-4}) مكغم .سم-2 وبمعامل ارتباط (0.9999) ، وحد كشف للطريقة (0.12) مكغم .مل$^{-1}$ وبمعدل انحراف قياسي نسبي افضل من (1.013%) .

طبقت الطريقة بنجاح لتقدير الليفو دوبا في مستحضراته الصيدلانية وكانت حدود الاسترداد المؤي مساوي الى (99.68 %).

Introduction :

The dopamine derivatives participate in the regulation of a wide variety of physiological functions in the human bod , L-Dopa, also known as levodopa is used as a first-line treatment for Parkinson’s disease, usually along and with carbidopa or benserazide[(1)] .The drug readily crosses the blood brain barrier and is decarboxylated to dopamine in the brain.

This occurs both in the peripheral circulation and in the central nervous system after levodopa has crossed the blood brain barrier. Activation of central dopamine receptors improves the symptoms of

Parkinson's disease; Parkinson's disease is one of the most difficult problems in the medical field. The cause of this disease is a significant depletion of dopamine due to the death of neurons which can produce dopamine in brain. It leads to tremor, muscle stiffness, bradykinesia, and so on[2]. Levodopa a precursor of dopamine, is an important neurotransmitter which is used for the medication of neural disorders such as Parkinson's disease. After administration, levodopa is converted into dopamine through enzymatic reaction catalyzed by dopadecarboxylase[3].

A vast number of methods have been developed for the analysis of these compounds. L-Dopa is estimated by LC-MS-MS[4], chemiluminescence[5], HPLC-DAD[6] , voltametry[7], and HPTLC[8]. NMR[9] and HPLC-MS[10], methods.

Also, several spectrophotometric methods are reported for the determination of L-Dopa using tris(1,10- phenanthroline) [11], p-nitro aniline[12], sulphanilamide[13], sulfanilic acid[14], 4-aminobenzoic acid[15], isoniazid[16], sodium metaperiodate[17], Cu(II)- neocuproine[18], chloranil[19], potassium ferricyanide[20], and 4-aminoantipyrine[21] as achromogenic reagent .

The proposed method is based on the reaction of the L-dopa drug with 4-AAP in the presence of sodium hydroxide as alkaline medium to form a red water soluble dye product which shows an absorption maximum at 519 nm .

Experimental

Apparatus:

- All spectral and absorbance measurement were carried out in a Double beam UV-Vis spectrophotometer-1800 . Equipped with a 1 cm quarts cell .

- Water bath (Lab. Companion , BS - 11) .

- Electronic balance (Sartorius AG GÖTTINGEN B2 2105 Gerrmany) .

- PH – Metter(PW- 9421) .

Reagents:

All chemicals used were of analytical-reagent grade .

-stock solutions from drug (100 $\mu g.ml^{-1}$) of L-dopa (Fluka) were prepared by dissolving (0.01)gm of L-dopa in distilled water and diluting to the mark in 100 ml volumetric flask . Working solutions were prepared by diluting the solution in distilled water .

- 4-aminoantipyrine(4-AAP) (0.30 M) stock solution was prepared by dissolving (6.97) gm of 4-AAP in 10 ml of ethanol and the solution made up to the mark in 100 ml volumetric flask with distal water .

- sodium hydroxide (NaOH) (2.00 M) stock solution was prepared by dissolving 8 gm of NaOH in distilled water and diluting to the mark in 100 ml volumetric flask and then standardization of this solution with standard solution of HCl .

procedure for pharmaceutical preparations :

dopal forte tablet and sienamat tablet :

10 tablets were grinded well and acertain portion of the final powder was accurately weighted to give an equivalent to about 10 mg of L-dopa was dissolved in distilled water . The

prepared solution transferred to 100 ml volumetric flask and made up to the mark with distilled water forming a solution of 100 µg.ml^{-1} concentration . The solution was filtered by using a Whatmann filter paper No. 42 to avoid any suspended particles .These solution were diluted quantitatively to produce a concentrations in the range of calibration curve .

Recommended procedure :

In to a series of 25 ml volumetric flask , transfer increasing volume of L-dopa solution (100 µg.ml^{-1}) to cover the range of calibration curve (0.20 – 30.00) µg.ml^{-1} ,

added 4.16 ml (0.05 M) of 4-AAP and shake well . Added 0.60 ml (0.05 M) of NaOH , dilute the solution to the mark with distilled water , and allow the reaction to stand for 30 min at room temperature (25 ^{o}c) . measure the absorption at(519 nm) against a reagent blank prepared in the same way but containing no L-dopa .

Results and Discussion :

Absorption spectra :

Levo-dopa drug react with 4-amino anti pyrine in the presence of sodium hydroxide as alkaline media to form an intense red colour product that can be measured spectrophotometrically at 519nm (Fig .1) .

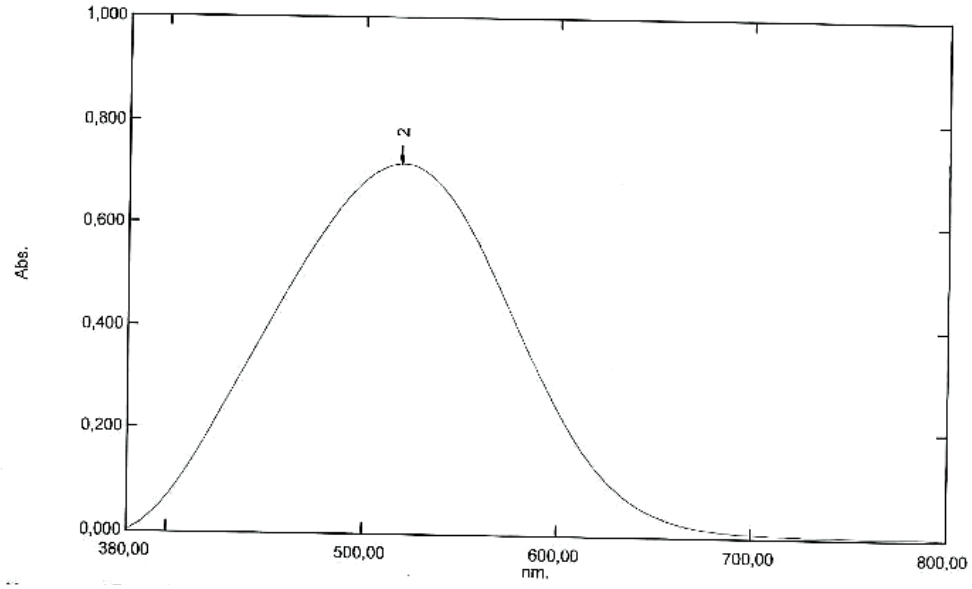
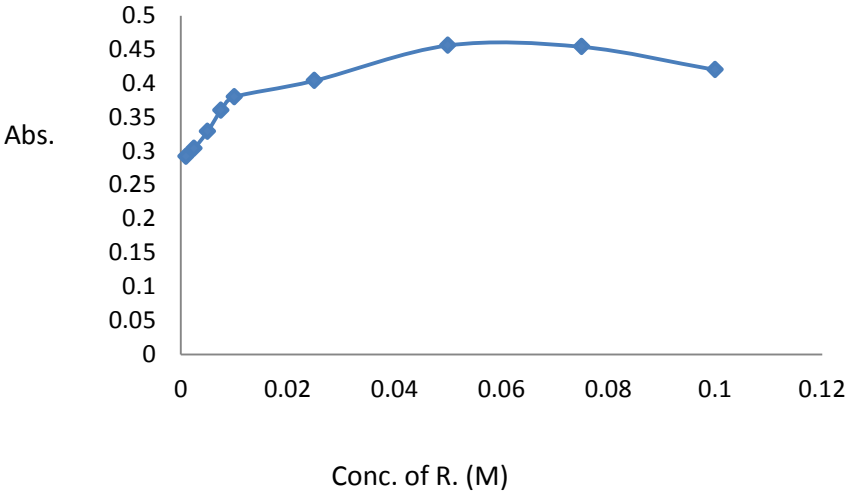


Fig (1) : Absorption spectra of (26 µg.ml^{-1}) of L-dopa with 4-AAP (0.05M) , and NaOH (0.05M) and allow the reaction to stand for 30 min at room temperature (25 ^{o}c) , (Abs.=0.717 at 519nm) and measured against blank solution .

The absorbance is directly related to the concentration of Levo-dopa drug and can be used for the colour product dye depends on the reaction conditions and was optimized as follows :

Effect of 4-aminoantipyrine (4-AAP) Concentration :

The effects of various concentration of 4-AAP were investigated . A Concentration of (0.05)M gave the highest absorbance at 519nm and was chosen for further use . The reslts are shown in Fig (2) and thus was chosen for further use .



Fig(2):Effect of 4-AAP Concentration on Absorption spectra of (16 µg.ml^{-1})of L-dopa

Effect of alkaline media type :

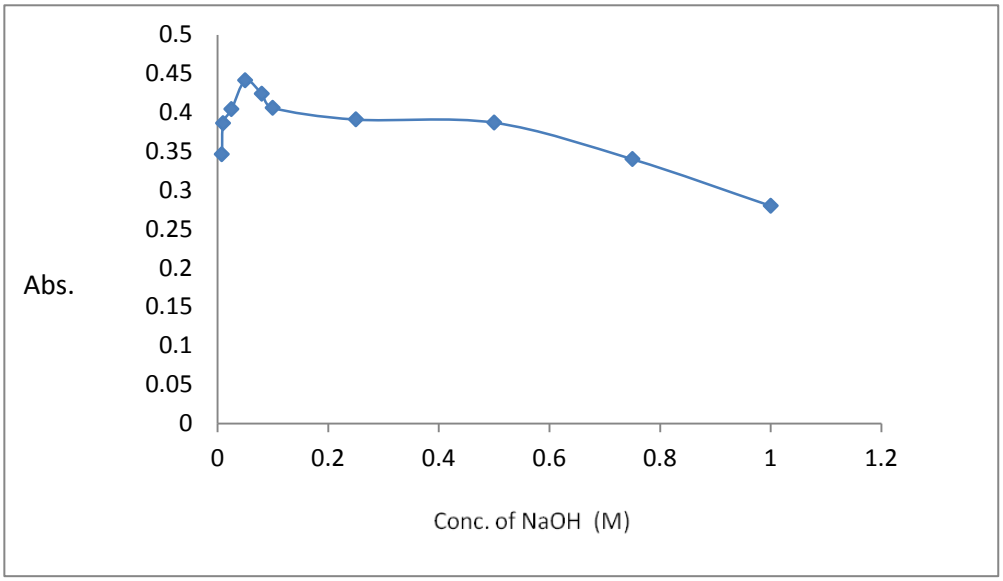
Levo-dopa drug react with 4-amino anti pyrine in the presence of alkaline medias ther fore the type of alkaline media is an obtained showed that sodium hydroxide gave the best absorbance as shown in Table(1) and was used in the recommended procedure .

Table (1) Effect of alkaline media type

Base (0.01)M	Abs.
NaOH	0.386
KOH	0.302
$Ca(OH)_2$	0.285
$Ba(OH)_2$	0.313
NH_4OH	0.318
Na_2CO_3	0.183
$NaHCO_3$	0.201

Effect of sodium hydroxide NaOH Concentration :

The effect of NaOH Concentration in the range of (0.8* 10^{-2} - 1)M was similarly studied . A Concentration of (0.05)M of NaOH give the higher absorption intensity at 519 nm for 16 µg . ml^{-1} of L-dopa and (0.05)M of 4-AAP.The results obtained are shown in Fig (3).



Fig(3) :Effect of NaOH Concentration on Absorption spectra of (16 µg.ml^{-1})of L-dopa

Order of addition :

The effect of order of addition on the absorption of red water soluble dye was studied . Table (2) , shows the order of addition could be followed ,

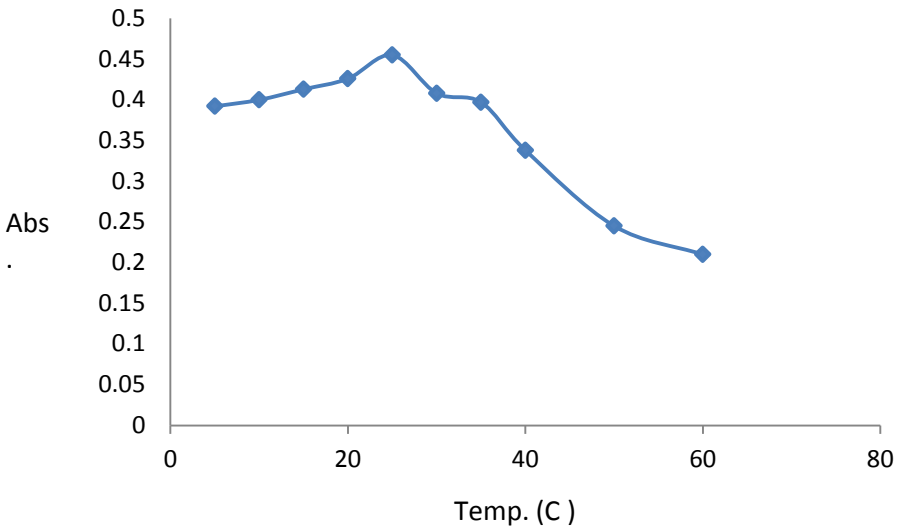
(Drug : 4-AAP : NaOH) . Due to gave the highest absorption and thus was chosen for further use .

Table (2) Effect of order of addition

Order of addition	Absorbance at λ max (519)nm
Drug : 4-AAP : NaOH	0.456
Drug: NaOH: 4-AAP	0.420
NaOH : 4-AAP : Drug	0.390
NaOH : Drug : 4-AAP	0.423
4-AAP : Drug : NaOH	0.410
4-AAP : NaOH: Drug	0.385

Effect of Temperature :

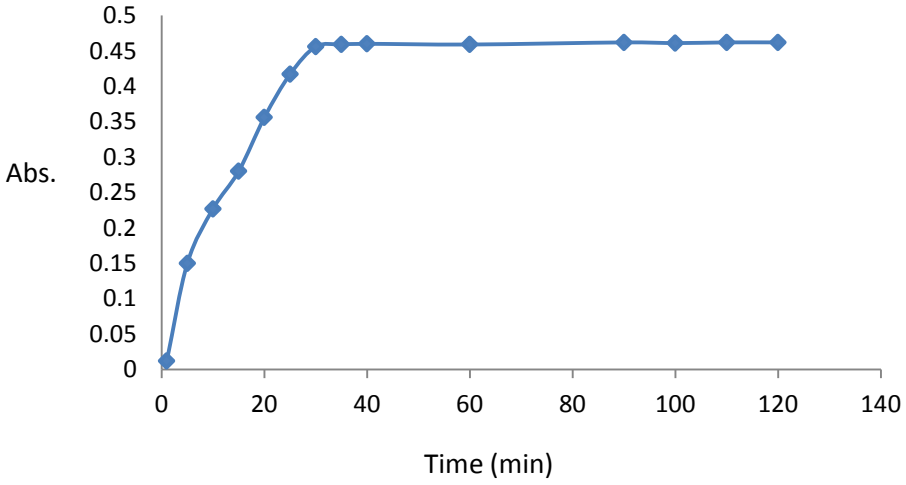
The effect of Temperature on the color intensity of the product was studied in practice the highest absorption was obtained when the colored product was developed at room temperature (25ºc) . as shown in Fig (4)



Fig(4) :Effect of Temperature on Absorption spectra of (16 µg.ml^{-1}) of L-dopa

Effect of Time :

The color intensity reached a maximum absorption after L-dopa has been reacted with 4-AAP and NaOH at 30 min . Therefore 30 min development time was chosen for further use . The results obtained are shown in Fig (5)



Fig(5) :Effect of Time on Absorption spectra of (16 µg.ml^{-1}) of L-dopa .

Calibration Curve :

Under the optimum conditions , a linear calibration graph for the determination of L-dopa was obtained over the concentration range of (0.2 - 30)µg.ml^{-1} . The linear regression equation for the determination of L-dopa is(Y=0.0268 X +0..0162) and correlation coefficient of 0.9999 the linear calibration graph is shown in Fig (6) .

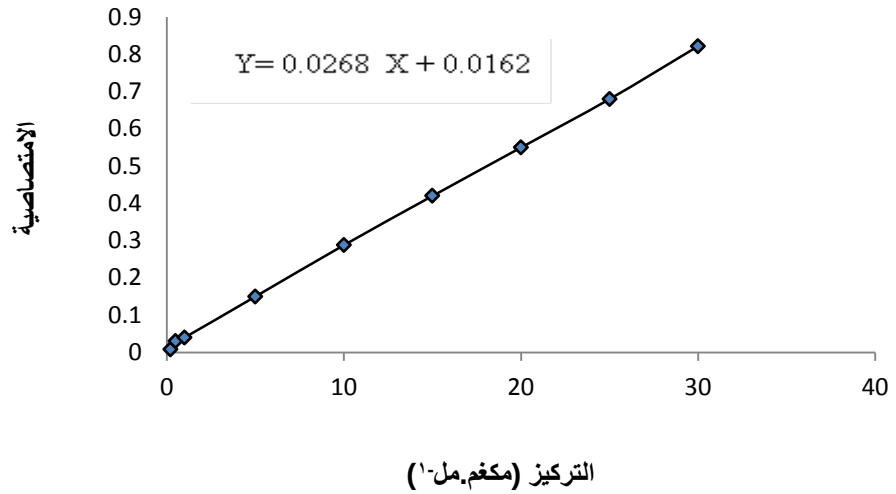
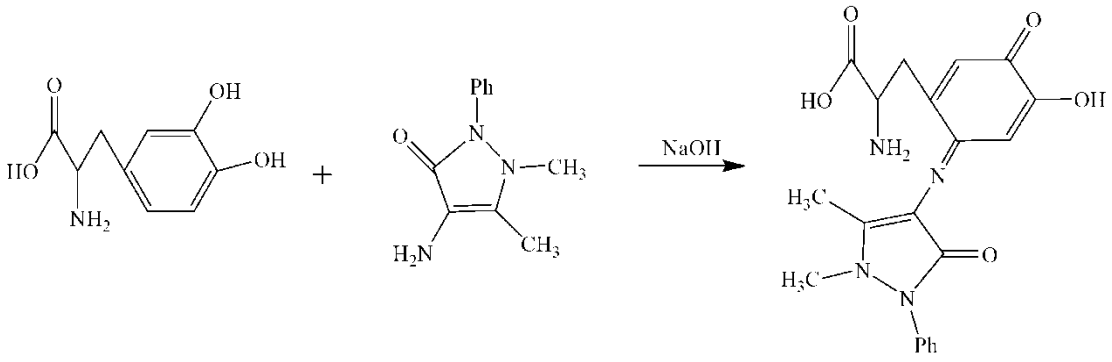


Fig (6) : calibration Curve for the determination of L-dopa .

Nature of the dye product :

The stoichiometry of the reaction between L-dopa and 4-AAP in alkaline media was investigated using the molar ratio method under the optimized conditions . The results obtained Fig (7) , show a 1:1 drugs to reagent product was formed .The formation of the dye may probably be occur as follows[22] :



levodopa 4-aminoanty pyrine Dye product

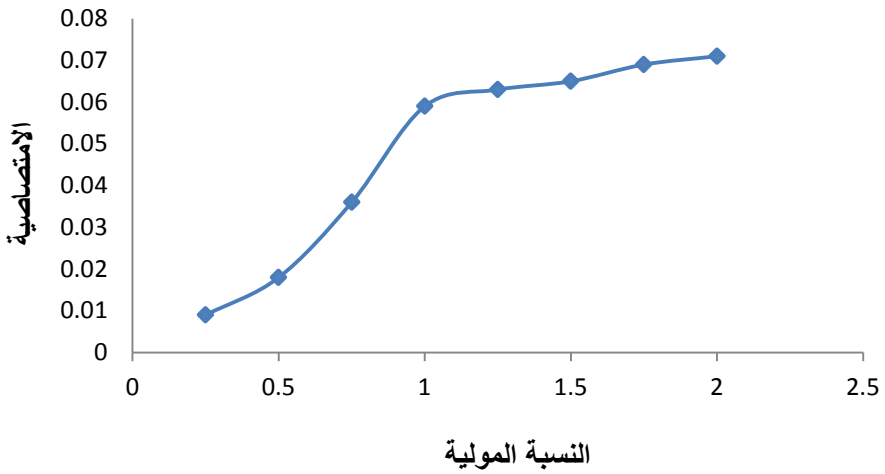


Fig (7) : Molar ratio of drug to reagent

Interference studies :

Several pharmaceutical preparations are associated with flavoring agents, diluents and excipients. Table (3) shows the effect of organic and inorganic interfering materials that may be present in pharmaceutical preparations.

Table (3) : Influence of excipients and additives as interfering species in the determination of L-dopa

Foreign compound	Recovary (%) of 500µg L-dopa per µg compound added				
	100	500	1000	2000	5000
Glucose	101.77	100.95	102.54	99.46	98.23
Lactose	99.28	99.89	101.74	101.32	102.1
Starch	100.19	100.23	101.36	99.80	98.36
Sucrose	102.05	100.38	101.39	101.45	100.60
Sodium chloride	101.16	101.28	102.12	102.4	98.43
EDTA	99.28	98.95	101.98	99.18	98.25
Citric acid	101.23	101.02	100.03	98.77	102.46
Magnesium setarate	99.18	99.38	99.35	100.61	98.12

*average of five determination .

Analytical Application :

The proposed method was applied for the determination of L-dopa drag in pharmaceutical preparations. Good accuracy and precision were obtained for the studied drugs . The results obtained were given in Tabel 1 which confirm Finally, the proposed method was compared successfully with the standard method Table(4). Statistical analysis , F – and T – test , reveals that there is no significant difference in precession and accuracy between the proposed and the official spectrophotometric methods .

Table (4) : Application of the proposed method for the determination of L-dopa in pharmaceutical preparations .

Drug sample	Amount of L-dopa µg.ml^{-1}		Proposed Method			Standard Method
	Taken	Found	RSD %*	Error *	Recovery*	Recovery %[22]
Pure Levodopa (Fluka)	5.00	5.60	1.30	0.60	99.40	100
Dopal Forte tablets	5.00	5.55	1.300	0.55	99.45	
	15.00	15.51	1.040	0.51	99.49	
	20.00	20.27	0.890	0.27	99.73	
Sienamt tablets	5.00	5.60	1.140	0.60	99.40	
	15.00	15.40	0.980	0.40	99.60	
	20.00	20.32	0.730	0.32	99.68	

*Average of five determinations .

Conclusion :

A simple ,accurate and sensitive spectrophotometric method was investigated for the determination of Levo-dopa in pure and in pharmaceutical formulations . The proposed method can be carried out with no need for further steps such as solvent extraction step , pH or Temperature control .

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