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SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF L-DOPA IN PHARMACEUTICAL FORMULATION USING 7-CHLORO-4-NITROBENZOXADIAZOLE (NBD-CL) AS A CHROMOGENIC REAGENT

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ABSTRACT

A new simple and sensitive spectrophotometric method for the determination of L-dopa has been developed. The method is based on the reaction of with NBD-Cl in alkaline media pH 12.0 to yield purple-brown colored products. L-dopa showed maximum absorbance at 28 nm and the product at 544 nm with linearity was observed in the concentration range of 1-12 µg/mL. The accuracy and precision of proposed method were satisfactory. The proposed method was successfully applied for analysis of L-dopa in its pharmaceutical formulation and a recovery percentage of 94.8% was optioned.

KEYWORD: spectrophotometric, L-dopa, Pharmaceutical formulation, 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl).

INTRODUCTION

L-dopa (L-3, 4-dihydroxyphenylalanine) is a chemical that is made and used as part of the normal biology of humans, some animals and plants. Some animals and humans make it via biosynthesis from the amino acid L-tyrosine. L-dopa is the neurotransmitters dopamine, norepinephrine (noradrenaline),and epinephrine (adrenaline) collectively known as catecholamines. L-dopa can be manufactured and in its pure form is sold as a psychoactive drug with the INN levodopa;trade names include Sinemet, Parcopa, Atamet, Stalevo, Madopar, Prolopa, etc. As a drug it is used in the clinical treatment of Parkinson's

disease and dopamine-responsive dystonia. (S)-2-Amino-3-(3,4-dihydroxyphenyl) propanoic acid.[1]

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. 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.[2, 3]
A number of analytical methods have been reported for the analysis of L-dopa in pharmaceutical formulation including LC-MS-MS [4], chemiluminescence [5], voltammetery [6], HPTLC [7] using a modified carbon nanotube Paste [8] and spectrophotometry.[9-12]

Spectrophotometry is probably the most convenient analytical technique for routine analysis because of its inherent simplicity, low cost and wide availability in quality control laboratories. The spectrophotometric methods previously reported for the determination of L-dopa.[10-11] These methods are associated with major drawbacks such as the need for multiple extraction steps, such as lack of sensitivity, tedious extraction procedures, heating, cooling and time consumption. In this paper new spectrophotometric method for the determination of L-dopa in pharmaceutical tablets that overcome these drawbacks is reported.

2-Chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD-CL) has been proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group.[13-26] The applications of NBD-CL for determination of pharmaceutical bearing amine group have been reviewed by Elbashir et al.[27, 28] The reaction between L-dopa and NBD-Cl has not investigated yet, therefore, the present study was devoted to investigate the reaction between NBD-Cl and L-dopa, and use this color reaction in the development of simple, rapid spectrophotometric method for determination of L-dopa in pharmaceutical formulation.

2. EXPERIMENTAL AND METHOD

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements.

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

- Electronic balance (Sartorius AG GÖTTINGEN B2 2105 Germany)
- pH – Metter (PW- 9421).

REAGENTS

L-dopa; was obtained from Aldrich chemical Co., St.Louis, USA. NBD-Cl 98%, was purchased from Sigma-Aldrich (Steinheim, Germany).

Preparation of standard and sample solutions

Stock standard solution of L-dopa (100 µg/mL)

An accurately weighed 0.005g standard sample was dissolved in water, transferred into a 50 mL standard flask and diluted to the mark with water and mixed well. The solution was freshly prepared.

7-Chloro-4-nitrobenzoxadiazole (NBD-Cl 0.024% w/v)

A weighed 0.024 g NBD-Cl was dissolved in water transferred into a 100 mL standard flask and completed to the mark. The solution was freshly prepared.

Tablets sample solution

Ten tablets were weighed accurately and pulverized. Then an accurately weighed amount equivalent to 0.0050 g was transferred into a 50 mL volumetric flask. The prepared solution was diluted quantitatively to obtain a suitable concentration for the analysis, and then the general procedure was followed as described below.

Assay procedures

Aliquots of solution were added to 10 mL volumetric flasks to give final concentrations of 1-12 µg/mL. Buffer solution (pH 12.0, 0.7 mL) was added followed by 2.0 mL solution (0.024%, w/v) NBD-Cl. There action was allowed to proceed at room temperature for 20 min after which the reaction mixture was made up to the mark with water and the absorbance measured at 544 nm against a blank similarly prepared.

Job's method

The Job's method of continuous variation [29] was employed. Master equimolar (1×10^{-4} M) aqueous solution of L-dopa and NQS were prepared. Series of 10 mL portions of the master solution of L-dopa and NBD-Cl were made up comprising different complementary proportions (1:9,…9:1, inclusive) in 10 mL volumetric flask and 0.7 mL of buffer solution

(pH 12.0) were added . The solution was further manipulated as described under the general recommended procedure Stability.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectrum of L-Doba was recorded against water (Figure 1), it was found that L-Doba exhibits a maximum absorption peak (λ_{max}) at 280 nm. Because of highly blue shifted λ_{max} of L-Doba, its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, derivatization of L-Doba red–shifted light-absorbing derivative was necessary. The reaction between L-Doba and NBD-Cl was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is purple-brown colored exhibiting λ_{max} at 544 nm, and the λ_{max} of NBD-Cl was 342 nm. The λ_{max} of L-Doba-NBD-CL derivative was red-shifted, eliminating any potential interference. Therefore, the measurements were carried out at 544 nm.

Optimization of Reaction Variables

Effect of pH

The influence of pH on the reaction of L-dopa with NBD-Cl was investigated by carrying out the reaction in buffer solution of varying pH values. The results revealed that L-dopa has difficulty to react NBD-Cl in acidic media (Figure 2). This was possibly due to the existence of the amino group of L-dopa in the form of salt, thus it loses its nucleophilic substitution capability. As the pH increased, the readings increased rapidly, as the amino group of L-dopa turns into the free amino group, thus facilitating the nucleophilic substitution. The maximum readings were attained at pH values of 12.0. At higher pH value, it decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction.

Effect of Reagent Concentration

The effect of NBD-Cl concentration on the reaction with L-dopa revealed that the reaction was dependent on the reagent concentration as the readings increased with the increase in the reagent concentration (Figure 3). The highest readings were attained at concentration 0.024% (w/v). For high precise values, further experiments were carried out using this concentration.

Effect of reaction time By following the reaction for various lengths of time it was found that the reaction went to completion over 20 min and a longer reaction time was not necessary Figure 4.

Effect of volume of buffer solution

The effect of volume of buffer solution on the absorbance was investigated in the range of 0.5–1.2 mL. The maximum and constant absorbance intensity was obtained in 0.7 mL Figure 5.

Composition of product

The continuous variation method of equivalent mole method was used to determine the composition of Product. The result is shown in Figure 6. As can be seen, the mole ratio of l-dopa and NBD-Cl of Product I is 1:1 . Based on the observation molar ratio, the reaction pathway was postulated to proceed as shown in Scheme 1.

Validation of the proposed methods

The validity of the methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to International Conference on Harmonization (ICH) [30, 31] States. Linearity, detection, and quantification limits by using the above procedure, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the analytical response to the concentration of L-dopa over the ranges cited in Table 1. Linear regression analysis of the data gave the following equation A = 0.1937+ 0.0644C, r = 0.9988 . Where A is the absorbance, C is the concentration of the drug (µg/ml), and r is the correlation coefficient.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH.[30, 31] The results are shown in Table 1. The limits of detection (LOD) were determined by establishing the minimum level at which the analyte can be reliably detected, and the results are also summarized in Table 1. LOQ and LOD were calculated according to the following equations:

LOQ =10s/b

LOD = 3:3s/b

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and b is the slope of the calibration curve.

Accuracy and precision

The accuracy and precision of the proposed method were determined at three concentration levels of L-dopa (within the linear range) by analyzing three replicate analyses on pure drug of each concentration. The percentage relative error as accuracy and percentage relative standard deviations (RSD) as precision for the results did not exceed 4.2 % as shown in Table 2, indicating the good reproducibility and repeatability of the method. This good level of precision and accuracy was suitable for quality control analysis of L-dopa in their pharmaceutical formulation.

Recovery

The recovery of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to a known concentration of the drug sample. The average percent recoveries obtained in range 102-96.1% Table 3.

Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the methods variables did not significantly affect the procedures. This provided an indication of the reliability of the proposed methods during routine work; recovery values were shown in Table 4.

Applications of the Method

The proposed method was applied to the pharmaceutical formulations of L-dopa; indicate the high accuracy of the proposed method for the determination of the studied drug. The proposed method has the advantage of being virtually free from interferences by excipients. The percentage was 94.8% (value is means of five determinations).

Table 1. Parameters for the performance of the proposed method

Parameter	L-dopa
λ_{max}, nm (Drug)	280
λ_{max}, nm(product)	544
Beer's law limits, µg/mL	1-12
Molar absorptivity, l/mol cm	$104.676*10^3$
Limit of detection, µg/mL	0.057
Limit of quantification, µg/mL	0.17
Regression equation, Y*:	
Intercept (a)	0.1936
Standard deviation of intercept	0.007995
Slope (b)	0.06436
Standard deviation of slope	0.001129
Correlation coefficient (r^2)	0.9987
Standard deviation	0.0111

Table 2. Precision results for the proposed method

Concentration taken µg/mL	Concentration found µg/mL	%±RSD
2	1.8	92.6±0.3
4	4.2	106±0.4
9	9.2	102±0.7

Table 3. Recovery of the proposed method

Sample No.	Sample contact µg/mL	Added µg/mL	Found µg/mL	%±RSD
1	1	1	2.007	101±0.016
2	"	5	6.1	102±0.008
3	"	6	6.7	96.1±0.07

Table 4. Robustness of the proposed method

Parameter	Recovery ± RSD*
pH 11.8 12.2	 104.30±1.1 103.30±0.8
Buffer volume 0.8 0.6	 98.40±1.2 98.1±2.3
Time (min) 18 22	 96.2±3.6 93.8±1.49
NBD-Cl concentration (w/v)% 0.022 0.026	 102±2.6 100±2.3

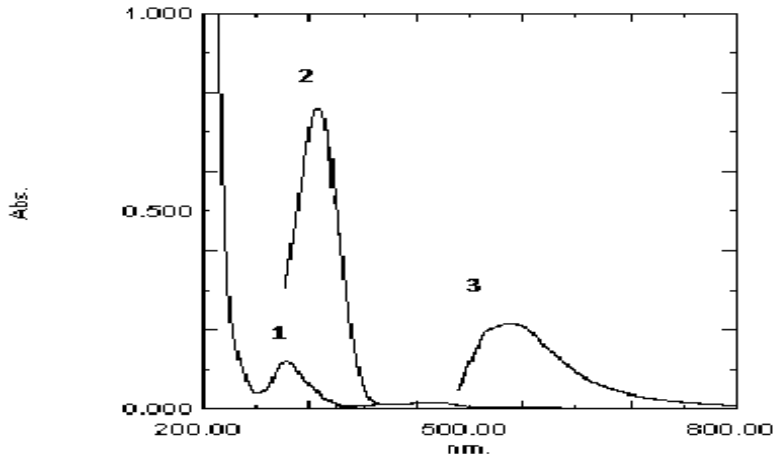


Figure 1 . (1) Absorption spectrum of L-dopa (10 µg/mL) against water. (2) Absorption spectrum of NBD-Cl (0.0012%) against water. (3) Absorption spectrum of reaction of L-dopa (2µg/mL) with NBD-Cl (0.024%).

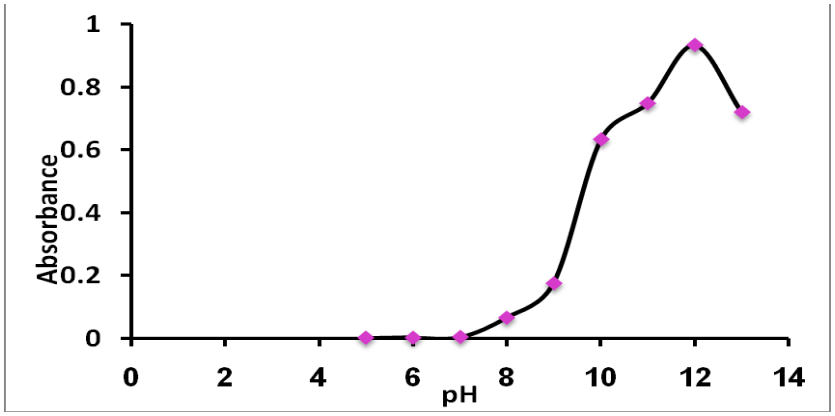


Figure 2. Effect of pH on the reaction of l-dopa with NBD-Cl. L-dopa (10.0 µ/mL): 1.0 mL; buffer solution of different pH values: 2 mL; NBD-Cl (0.024 %, w/v): 2.0 mL; reaction time:15 min.

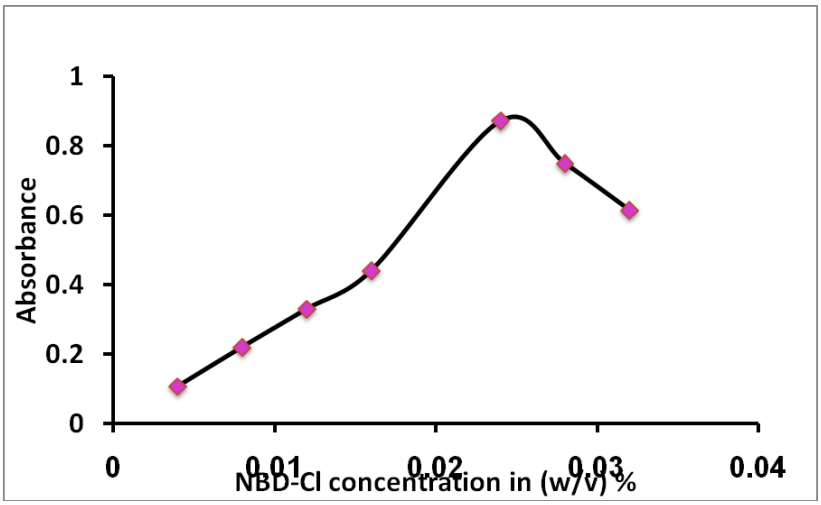


Figure 3. Effect of NBD-Cl concentrations on the reaction of l-dopa with NBD-Cl .l-dopa (10.0 µ/mL): 1.0 mL; NBD-Cl (0.02%): 2.0 mL ;buffer solution (pH 12): 0.7 mL ; reaction time:15 min.

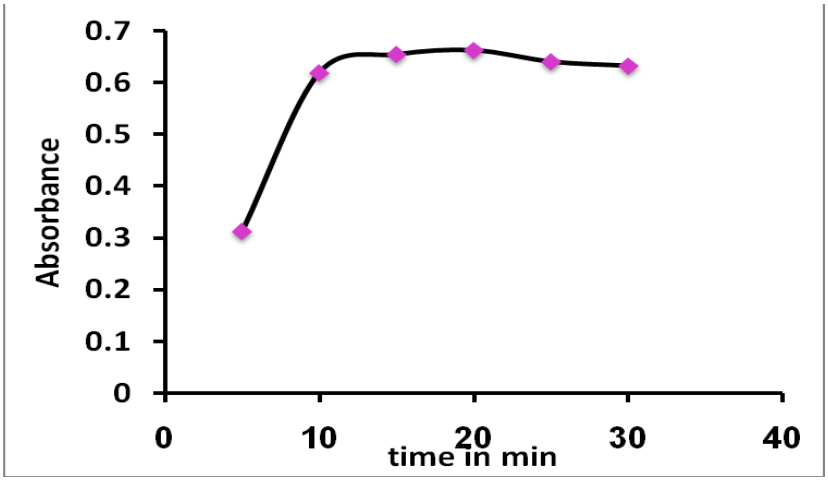


Figure 4. Effect of standing time on the reaction of L-dopa with NBD-Cl. l-dopa (10.0 µ/mL) ; NBD-Cl(0.024 %): 2 mL; buffer (pH 12.0):2 mL.

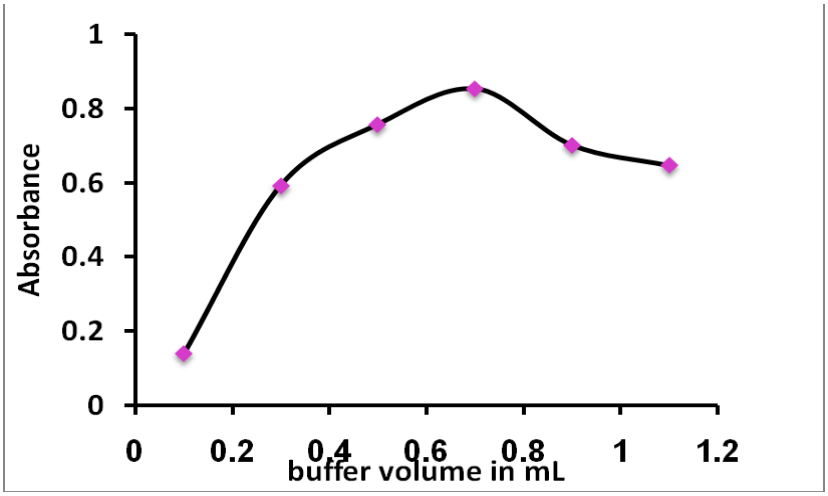


Figure 5. Effect of the volume of the buffer on the reaction of L-dopa with NBD-Cl. L-dopa (10.0 µ/mL):1.0 mL; NBD-Cl (0.02% w/v): 2.0 mL; buffer solution (pH 12.0); reaction time: 15 min.

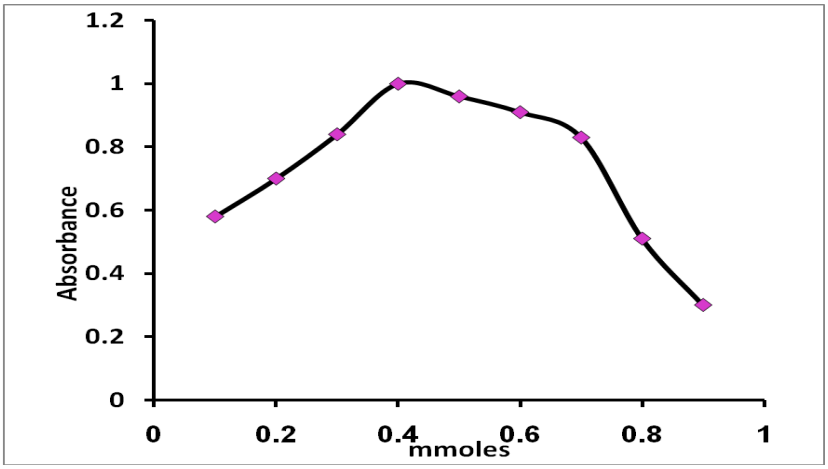
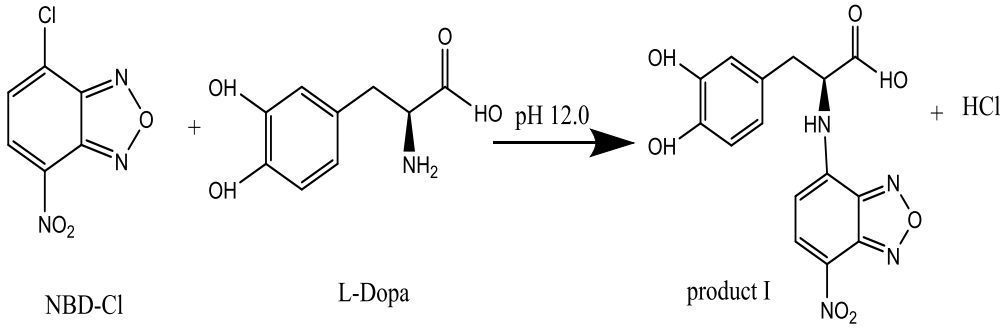


Figure 6 .Determination of Product formation by continuous variation method. VR: NBD-Cl($4x10^{-4}$ M); VD: L-dopa ($4x10^{-4}$ M); VR + VD = 10 mL.



Scheme 1. Reaction pathway of L-dopa with NBD-Cl

CONCLUSIONS

The proposed method for the determination of L-dopa in pharmaceutical formulations is based on the reaction of L-dopa with NBD-Cl. The proposed method was optimized and validated for precision and accuracy. The present work has the advantage of having simple operation, high sensitivity, repeatability, and reproducibility. In addition, the proposed method has a linear dynamic range of 1–12.0µg/mL with a detection limit of 0.057µg/mL. Hence, the proposed method can be used for routine quality control analysis of L-dopa in industries, research laboratories, and hospitals.

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