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Spectrophotometric methods for the determination of L-tyrosine in pharmaceutical formulations

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Abstract : New simple and sensitive spectrophotometric method has been developed and validated for the determination of L-tyrosine in pharmaceutical formulations. The spectrophotometric method was based on the reaction of L-tyrosine with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) in an alkaline medium (pH 10.0) to form an orange product absorbers at 388 nm. The variables affecting the reaction of L-tyrosine with NBD-Cl was carefully studied and optimized. Under the optimum reaction conditions, good linear relationships were found between the readings and the concentrations of L-tyrosine in the ranges 10-50 μg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 2.85 μg/mL and 8.6 μg/mL, respectively. The method was successfully applied to the determination of L-tyrosine in its pharmaceutical formulations. **© Global Scientific Inc.**

Keywords : Spectrophotometric; L-tyrosine, Pharmaceutical formulation, 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl).

INTRODUCTION

Tyrosine is one of the 22 amino acids that are used by cells to synthesize proteins. It is a non-essential amino acid with a polar side group. Tyrosine is an amino acid precursor of important neurotransmitters such as dopamine[1]. Tyrosine is indispensable for humans. It maintains a positive nitrogen balance[2] and its absence could produce albinism, hypochondria, or depression. In contrast, high tyrosine concentration in culture medium increases sister chromatid exchange[3].

A number of methods have been developed for the analysis of L-tyrosine, including chromatographic methods[4-7], spectrophotometric methods[8], fluorometric method[9] and electrochemical method[10].

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[11-26]. 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

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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.

MATERIAL AND METHOD

Apparatus

All of the spectrophotometric measurements were made with a Double beam UV 1800 ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells (SHIMADZU Japan), sensitive electronic balance was used for weighing the chemicals.

Materials

7-Chloro-4-nitrobenzoxadiazole (NBD-Cl) and L-tyrosine were obtained Aldrich chemical Co., St. Louis, USA).

Preparation of standard and sample solutions

L-tyrosine (200 µg/mL)

200 µg/mL of L-tyrosine was prepared by dissolving 0.0050 g of L-tyrosine in 10 mL 0.01M HCL and transfer the solution in to 25 mL volumetric flask. The solution is completed to the mark by distilled water. The solution is freshly prepared.

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

A weighed 0.040 g NBD-Cl was dissolved in water transferred into a 100 mL standard flask and diluted to the mark with water and mixed well 30 mL of this solution was transferred to 50 mL volumetric flask then complete the volume with distilled water to the mark. The solution was freshly prepared.

Preparation of sample solution

Weight equivalent to 0.0025g L-tyrosine was dissolved in 0.01M HCL. The solution was transfer in 25 mL volumetric flask and then contents were made up to volume with distilled water.

Assay procedure

Aliquots of solution were added to 10 mL volumetric flasks to give final concentrations of 10-50 µg/mL. Buffer solution (pH 10.0, 1 mL) was added followed 2 mL of NBD-Cl solution (0.024%, w/v). There action was allowed to proceed at 70 C for 25 min after which the reaction mixture was made up to the mark with water and the absorbance measured at 388 nm against a blank similarly prepared.

Job's method

The Job's method of continuous variation[29] was employed. Master equimolar (1×10^{-3} M) aqueous solution of L-tyrosine and NBD-Cl were prepared. Series of 10 mL portions of the master solution of L-tyrosine and NBD-Cl were made up comprising different complementary proportions (1:9,…9:1, inclusive) in 10 mL volumetric flask and 2.0 mL of buffer solution (pH 10.0) were added. The solution was further manipulated as described under the general recommended procedure Stability.

RESULTS AND DISCUSSION

Absorption spectra

L-tyrosine exhibits maximum absorbance (λ_{max}) at 283 nm Figure 1. Being in the ultraviolet, absorbance at this wavelength is susceptible to interference from co-extracted excipients in the tablet formulation. Accordingly derivatization of L-tyrosine to produce a chromophore absorbing more in the visible region was appropriate. L-tyrosine contains a primary aliphatic amino group, which is suitable for derivatization by NBD-Cl, an analytical chromogenic reagent for the determination of primary and secondary amines[11–28]. L-tyrosine was found to react with NBD-Cl under the experimental conditions to form an orange colored product exhibiting λ_{max} at 388 nm (Figure 1). Under the optimum reaction conditions the absorbance was found to obey the Beer-Lambert law.

Effect of pH

The absorbance due to the product increased rapidly with increasing pH. Maximum absorbance was attained at pH10.0, and then decreased. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction. On this basis, a pH of 10.0 was selected for the reaction Figure 2.

Effect of temperature

Keeping all conditions unchanged, the influ-

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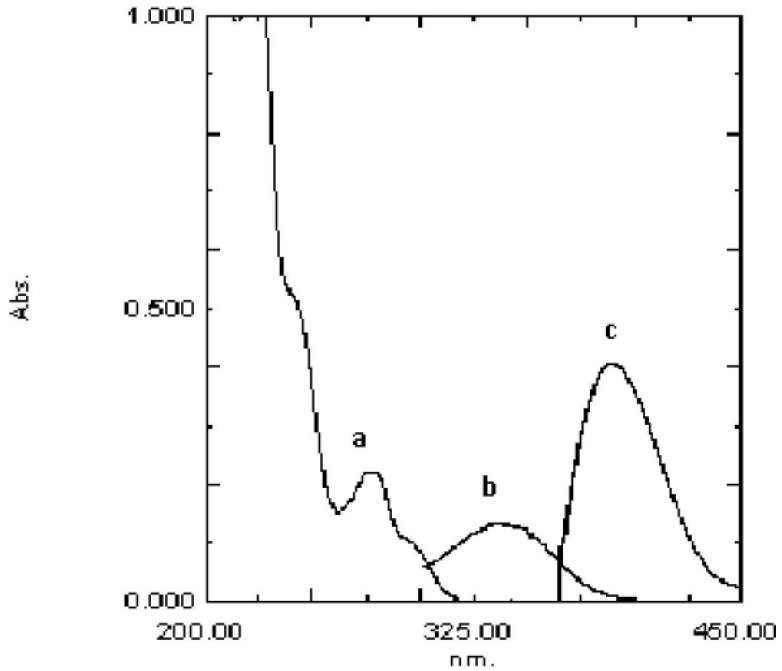

Figure 1 : (a) Absorption spectrum of L-tyrosin (25 µg/mL) against water, (b) Absorption spectrum of NBD-Cl (0.00048%) against water, (c) Absorption spectrum of reaction of L-tyrosine (20 µg/mL) with NBD-Cl (0.024%)

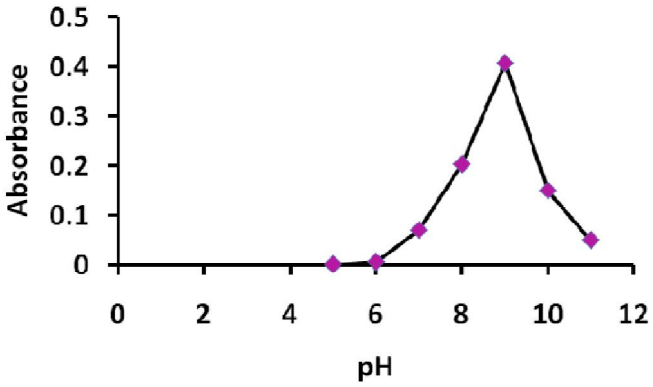

Figure 2 : Effect of pH on the reaction of L-tyrosine with NBD-Cl, L-dopa (20 µg/mL): 1.0 mL; buffer solution of different pH values: 2 mL; NBD-Cl (0.024%, w/v): 2.0 mL; reaction time:15 min.

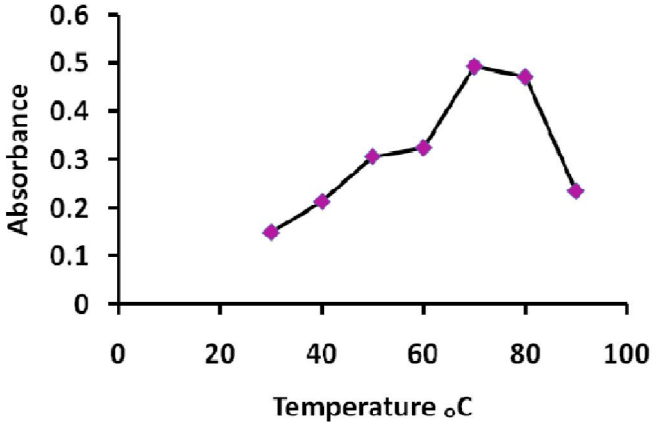

Figure 3 : Effect of temperature on the reaction of L-tyrosine with NBD-Cl. L-tyrosine (20 µg/mL):1.0 mL; NBD-Cl (0.0024%): 2.0 mL; buffer solution (pH 10.0): 1.0 mL; reaction time: 20 min.

ences of temperature on the absorbance of the solution were studied. It was found that the absorbance of solution was maximal at 70 ºC. Then there is a decrease with increasing temperature. In order to make the determination of L-tyrosine sensitive 70 ºC was chosen as the optimum reaction condition Figure 3.

Effect of reaction time

By following the reaction for various lengths of time it was found that the reaction went to completion over 25 min and a longer reaction time was not necessary Figure 4.

Effect of reagent concentration

The effect of reagent concentration was tested by using various concentrations solution of reagent with 1 mL of 20 µg/mL of tested drug. The results showed that 2 mL of 0.024%w/v reagent solution were sufficient for the production of maximum and reproducible color intensity of the investigated complex. Further excess of the reagents have no considerable effect on the absorbance Figure 5.

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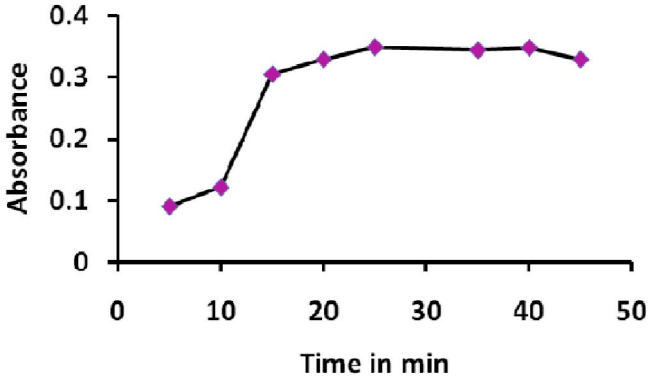


Figure 4 : Effect of standing time on the reaction of L-tyrosin with NBD-Cl. L-tyrosine (20 µg/mL) :1.0 mL; NBD-Cl (0.024%):2 mL; buffer (pH 10.0):1 mL.

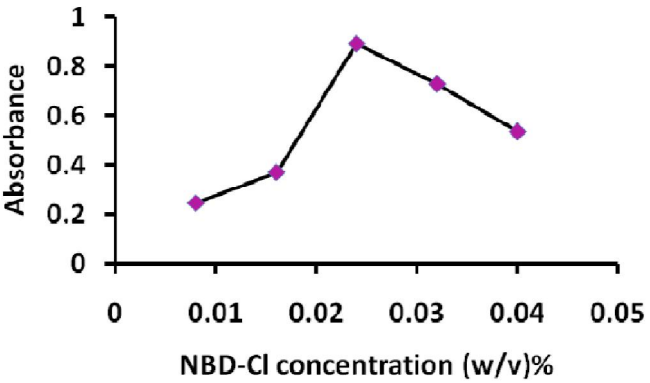


Figure 5 : Effect of NBD-Cl concentrations on the reaction of l-tyrosin with NBD-Cl. L-tyrosine (20 µg/mL): 1mL; NBD-Cl 2.0 mL; buffer solution (pH 10):1.0 mL; reaction time:20 min.

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-tyrosine 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] guidelines.

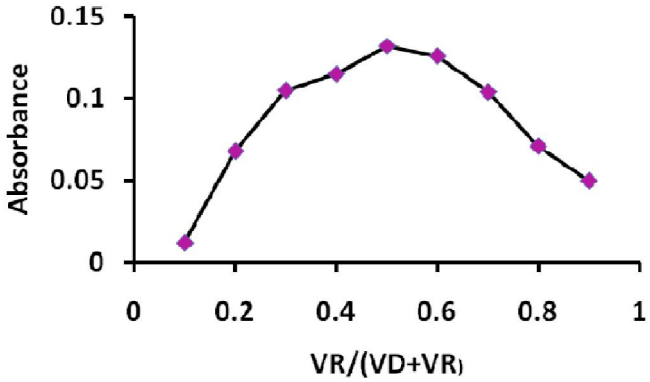


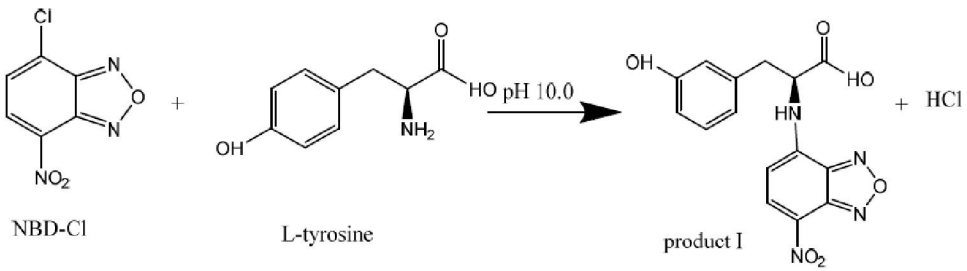
Figure 6 : Determination of Product formation by continuous variation method. VR: L-tyrosine (4x10Ét M); VD: NBD-Cl (4x10Ét M); VR + VD = 10 mL.

Linearity, detection, and quantification limits

By using the above procedures, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the analytical response in the method to the concentration of the drugs over the range cited in TABLE 1. Linear regression analysis of the data gave the following equations A = 0.0098C +0.4231, r = 0.996. 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



Scheme 1 : The reaction pathway of L-tyrosine with NBD-Cl

TABLE 1 : Parameters for the performance of the proposed method

Parameter	Value
λ_{max}, nm (Drug)	283
λ_{max}, nm(product)	388
Beer's law limits, µg/mL	10-50
Molar absorptivity, l/mol cm	58.5606*10?
Limit of detection, µg/mL	2.85
Limit of quantification, µg/mL	8.6
Regression equation, Y*:	
Intercept (a)	0.4231
Standard deviation of intercept	0.000358
Slope (b)	0.00977
Standard deviation of slope	0.0118
Correlation coefficient (r^2)	0.996
Standard deviation	0.0113

TABLE 2 : Precision results for the proposed method

Concentration taken µg/mL	Concentration found µg/mL	%±RSD
10	8.03	80.3±3.4
20	19.4	97.29±0.2
40	40.9	102±1.45

TABLE 3 : Recovery of the proposed method

Sample No.	Sample concentration µg/mL	Added µg/mL	Found µg/mL	%±RSD
1	10	10	19.1	95.7±2.6
2	"	20	31.3	104.6±0.001
3	"	30	38.2	95.5±2.1

TABLE 4 : Robustness of the proposed method

Parameter	Recovery ± RSD*
Recommended conditions	94.8±0.07
pH	
9.8	94.7±0.05
10.2	95.7±0.01
Temperature	
72	100±2.4
68	86.58±1.1
Time (min)	
23	100±1.2
27	96.27±1.3
NBD-Cl concentration (w/v)%	
0.0242	104.5±0.73
0.0238	104.5±4.3

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

The accuracy of the method was evaluated by performing three replicate analyses on pure drug solution at three different concentration levels (within the working range). Percentage relative standard deviation (RSD %) as precision as accuracy of the proposed spectrophotometric methods were

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calculated. The relative standard deviation (RSD) values were less than 2% in all cases, indicating good repeatability of the suggested method TABLE 2.

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 104.6-95.5%, TABLE 3.

Robustness

For the evaluation of the method robustness, some parameters were interchanged; pH, reagent concentration, temperature, reaction time. The capacity remains unaffected by small deliberate variations, TABLE 4.

Applications of the proposed methods

It is evident from the abovementioned results that the proposed methods gave satisfactory results with L-tyrosine in its bulk. Thus its pharmaceutical formulations were subjected to the analysis of their L-tyrosine contents by the proposed and the official methods. The percentage was 94.8% (value is means of five determinations).

CONCLUSIONS

The developed spectrophotometric method is precise, accurate and sensitive. No interference from the frequently encountered excipients and additives. Statistical analysis proves that the method could be applied for the analysis of the studied drugs in their pure forms and in pharmaceutical formulations.

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