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L-Tryptophan Measurement using Spectrophotometry and Spectrofluorometry in Pharmaceutical Formulations Containing 1-Chloro-4 Nitrobenzofurazon

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Annotation: For detecting L-tryptophan in pharmaceutical formulations, a novel, simple, and extremely accurate spectrophotometric and spectrofluorometric approach has been developed and shown to be successful. The production of an orange-colored molecule that absorbs at a wavelength of 405 nanometers when L-tryptophan and 1-chloro-4-nitrobenzofurazan (NBF-Cl) react in an alkaline medium (pH 10.5) served as the foundation for the spectrophotometric and fluorometric procedures. The variables affecting L-tryptophan's reaction to NBF were carefully investigated and improved. Under optimal reaction conditions, good linear correlations between the readings and the L-tryptophan concentrations in the range of 12 to 60 µg /mL were found. 8.6 and 2.85 µg/mL were found to be the limits of quantitation (LOQ) and detection (LOD), respectively. This method proved successful in identifying L-tryptophan in its pharmacological forms.

Keywords: L-tryptophan, Spectrofluorometric, 1-Chloro-4-Nitrobenzofurazon.

Introduction

Tryptophan is an essential amino acid that comes from either plant or animal sources because the

body cannot generate it and must get it through food. Tryptophan is regarded as one of the amino acids, which are the building blocks of protein, and it serves a variety of vital purposes, including growth in infants and nitrogen balance in adults [1,2]. Niacin, which is necessary for the synthesis of the neurotransmitter serotonin, is also produced using it. The orientation of the molecule is the only distinction between the two forms of tryptophan, which are L-tryptophan and D-tryptophan. Certain meals and dietary supplements are good sources of tryptophan [3]. Tryptophan has numerous applications, including the following: support for managing premenstrual syndrome symptoms like mood swings and exhaustion, assistance in stopping smoking, alleviation of teeth grinding, enhancement of sports performance, support for the treatment of depression and sleep issues like insomnia. aiding in the treatment of ADHD (attention deficit hyperactivity disorder), Improvements in pain tolerance, emotional health, and support for bipolar illness treatment [4,5].

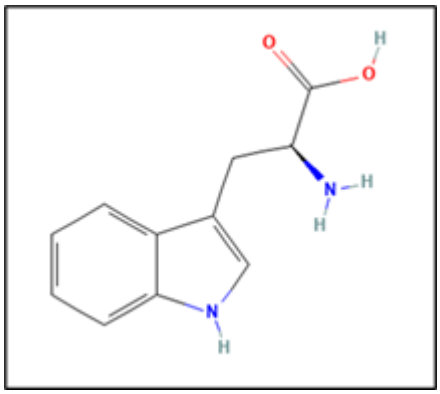
Numerous techniques, such as chromatography, spectrophotometric, fluorescence, and electrochemical approaches, have been developed for the analysis of L-tryptophan [5]. The effectiveness and sensitivity of 4-chloro-7-nitrobenzofurazan (NBF-Cl) as a derivatizing agent for the spectroscopic examination of pharmacological formulations with a primary or secondary amine group has been demonstrated. The uses of NBF for identifying the amine group in pharmacological formulations have been examined by several researchers [6,7]. The current study was devoted to examining the interaction between NBF and L-dopa, as well as using this chromatic interaction to develop a quick and easy spectroscopic method for determining L-dopa in pharmaceutical preparations, Also, on the other hand, since the interaction between these compounds has not been studied yet (NBF-Cl and L-dopa).

MATERIAL AND METHOD

The investigation was carried out in the Chemistry Laboratory of the College of Dentistry at the University of Karbala. A double beam UV-1800 spectrophotometer for visible and ultraviolet light, fitted with corresponding quartz cells that had a 1 cm diameter (SHIMADZU Japan), was used for all spectral measurements. Chemicals were weighed using a sensitive electronic balance. L-tryptophan and 1-chloro-4-nitrobenzofurazan (NBF-Cl) were acquired from Aldrich Chemicals, located in St. Louis, USA.

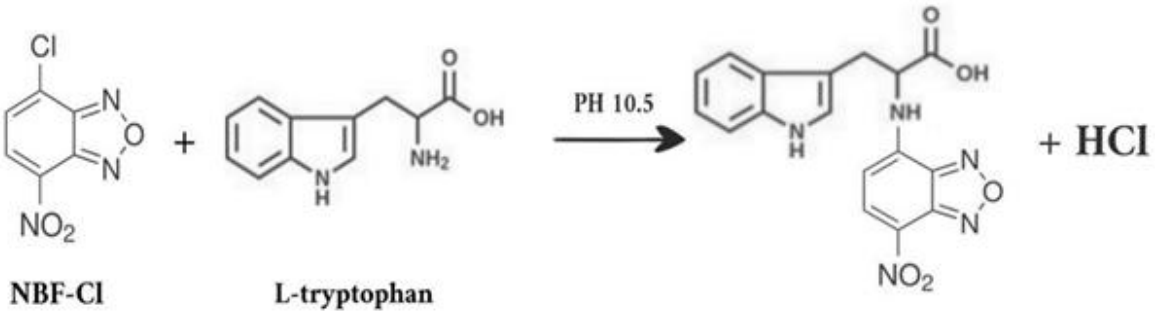
Preparing Samples and Standard Solutions

L-Tryptophan solution was prepared, by dissolving 0.050 grams of L-tryptophan in 25 milliliters of 0.01M hydrochloric acid, a solution containing (250 µg/mL) of L-tryptophan was created. The solution was then transferred to a 100 milliliter volumetric flask. Distilled water is added to the solution until the indicated line is reached. The solution is made from scratch. An NBF solution weighing 0.050g was dissolved in water, moved to a 200mL volumetric flask, and diluted with water until the desired level was reached before thoroughly mixing [9]. A 100mL volumetric flask was filled with 50mL of this solution, and the remaining volume was filled with pure water. The answer was prepared.



Scheme 1. The structure of L-tryptophan

Samples were prepared for measurement at the following concentrations and methods for the most audacious analyses: dissolved 0.0050g of L-Tryptophan in 0.02 M hydrochloric acid. After the solution was moved to a 50 mL volumetric flask, distilled water was added to adjust its volume. To get final concentrations between 10 to 50 µg /mL, To prepare the solution at different concentrations and with high accuracy in 10 ml volumetric flasks, small amounts of the solution were added. After adding 1mL of the buffer solution (pH 10.5), 2 mL of the NBF-Cl solution (0.025%, w/v) was added. After allowing this reaction to continue for 30 minutes at 75°C, water was added to the reaction mixture until it reached the desired level [10]. The absorbance at 405 nanometers was then measured against a sample that had been prepared in the same manner.



Scheme 2. The reaction pathway of L-tryptophan with NBF-Cl

RESULTS AND DISCUSSION

The Spectra of absorption of L-Tryptophan, the maximum absorption (λ_{max}) of L-Tryptophan is observed at 285 nanometers (Figure 1). Because it falls within the ultraviolet spectrum, excipients that are frequently taken from the tablet that needs to be made may interfere with its absorption at this wavelength. Therefore, it was suitable to derivatives L-Tryptophan to create a chromophore that absorbs more in the visible range. A primary aliphatic amino group found in L-Tryptophan can be derivatives using NBF-Cl, an analytical chromogenic reagent used to identify primary and secondary amines.

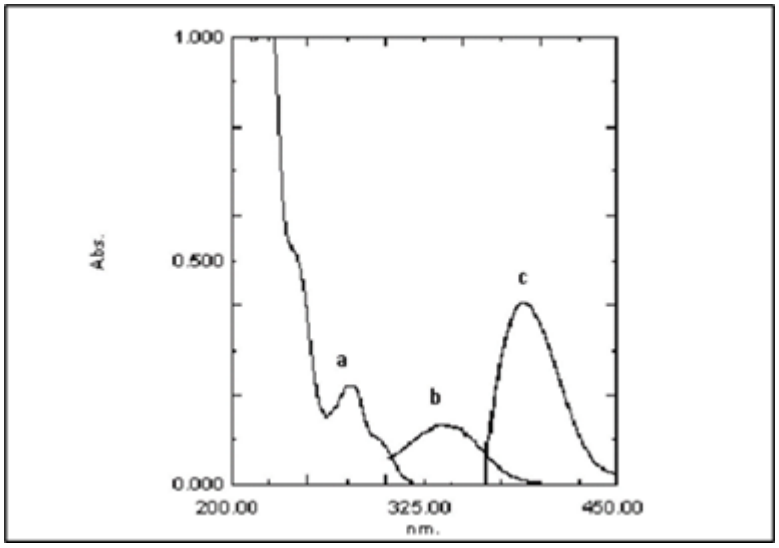


Figure 1: The absorption spectra of L-tryptophan (28 µg/mL) against water, NBF-Cl (0.00052%) against water, and L-tryptophan (25 µg/mL) reacting with NBF-Cl (0.024%) are shown in (A) and (B) respectively.

The effects of temperature on the solution's absorbance were examined while maintaining all other parameters constant. It was discovered that the solution's absorption was at its highest at 80 ^{o}C. Then, when the temperature rises, there comes a decline. As the ideal reaction condition, 80 ^{o}C was selected to determine the L-tryptophan sensitivity. As the pH increased the product's

absorbance rapidly increased [11]. At pH 10.5, the maximum absorbance was reached, after which it declined. This was most likely caused by an increase in the hydroxide ion concentration that inhibits the process. A pH of 10.5 was chosen for the reaction as showed in **Figure 2** based on this.

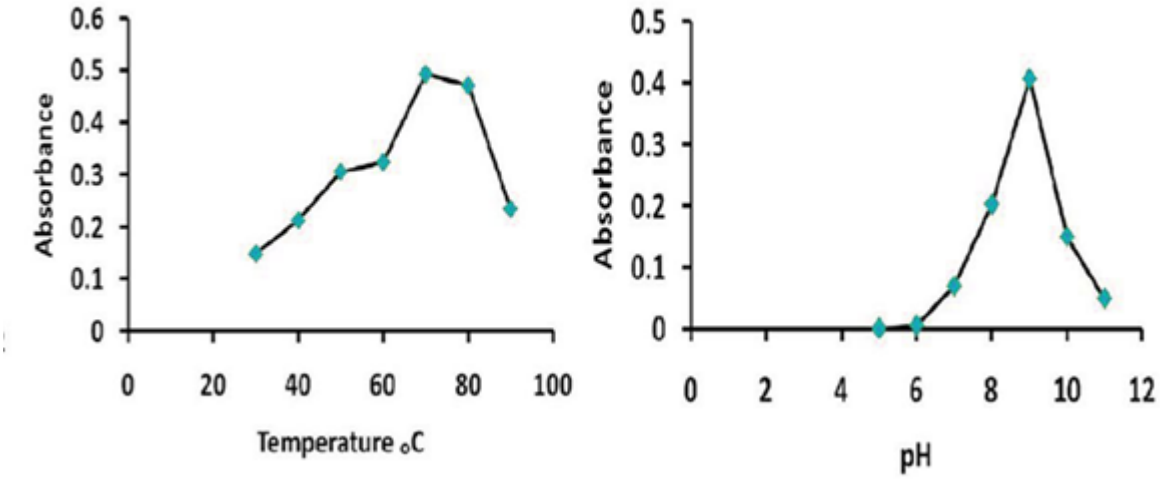


Figure 2: temperature's impact on L-tryptophan's interaction with NBF. L-dopa (20 µg/mL): 1.0 mL; L-tryptophan (20 µg/mL): 1.0 mL; NBF (0.0052%): 2.0 mL; and the impact of pH on the reaction between L-tryptophan and NBF

The findings of performance, accuracy, Robustness and recovery for the recommended method

Three identical analyses were conducted on a medication solution that is pure at three distinct concentration levels (within the operating range) in order to assess the method's accuracy. The accuracy of the suggested photometric spectrophotometric techniques was determined by calculating the relative standard deviation (RSD)as showed in **Table .1**. The determined. The suggested method's strong repeatability was demonstrated by the RSD values, which were consistently less than (3%). As the second table shows, **Table .2**. Through the use of the usual addition procedure, the suggested method was recovered [12]. A known concentration of the drug sample was mixed with varying amounts of standard solution. Table .1 shows that the average recovery percentages varied between (106.4 and 94.6%). Some factors, including pH, reagent concentration, temperature, and reaction time, were changed in order to assess the method's robustness. Intentional little adjustments have no effect on the capacitance, As the **Table .2** shows. The data above make it evident that using L-tryptophan in the formulation of the suggested approaches produced good outcomes [13,14]. Therefore, the suggested and official procedures were used to analyze the L-tryptophan concentration of its pharmaceutical formulations. The average of five estimates yielded 95.4% as the outcome.

Table .1 Performance indicators for the proposed technique

Objectives that used	Value
$\lambda_{max\ 1}$	285 nm
$\lambda_{max\ 2}$	405 nm
Molar absorptivity	$60.4505*10^{l-}$ mol/ cm
detection Limit	2.75 µg/mL
quantification Limit	9.2 µg/mL
Beer's law	15-60 µg/mL
Slope	0.00966
Intercept	0.4321

Standard deviation of slope	0.0127
Standard deviation of intercept	0.000365
Standard deviation	0.0122
Correlation coefficient (r^2)	0.989

Utilization of the suggested techniques and the limits of linearity, detection, and quantification

The data above make it clear that the suggested techniques produced good outcomes when using bulk L-tryptophan. Therefore, both the approved and suggested procedures were used to analyze the L-tryptophan content of its medicinal formulations. The percentage, which is the mean of five calculations, was 95.4%. Linear regression equations were produced by applying the aforementioned techniques. As the results showed in the regression graphs, a linear relationship was shown between the analytical response to the results in terms of the method and the drug concentration. As shown in Table 1. The following equations were obtained from the data's linear regression analysis: A = (0.0097 + 0.5123), (r = 0.989). where r is the correlation coefficient, C is the drug concentration (µg/mL), and A is the absorbance. By determining the lowest concentration that may be quantified in accordance with ICH, the quantification limit (LOQ) was determined [15, 16]. TABLE .1 displays the findings. TABLE .1 provides a summary of the findings. The detection limits (LOD) were established by determining that the lowest level of concentration (µg/mL) at which the analyses may be consistently detected. The following formulas were used to determine LOQ and LOD, LOQ is 10s/b, LOD is 3:3s/b [17].

Table .2 Accuracy results for the proposed method

Con. found µg/mL	Con. taken µg/mL	% ± RSD	concentration	Add con.	Found con.
9.02	5	75.3 ± 4.3	5	10	19.8
18.9	10	102.29 ± 0.3	10	20	32.5
39.9	20	105 ± 2.35	20	30	40.2
40.3	30	110 ± 3.45	30	40	47.8
43.5	40	112 ± 2.25	40	50	58.2

The influence of reaction time and reagent concentration

A solution of reagent at different concentrations was used with (1mL) from the solution that used with (20 µg/mL) of the tested drug to examine the effect of reagent concentration. The findings demonstrated that 2 milliliters of a (0.026 w/v) reagent solution that prepared already were adequate to produce the highest and most consistent color intensity that measured for the complex under investigation [18,19]. The absorbance is not significantly impacted by further reagent excess. Following the reaction for different amounts of time revealed that it took 25 minutes for it to complete and that a longer reaction time was not required [20]. The result is shown in Figure 3.

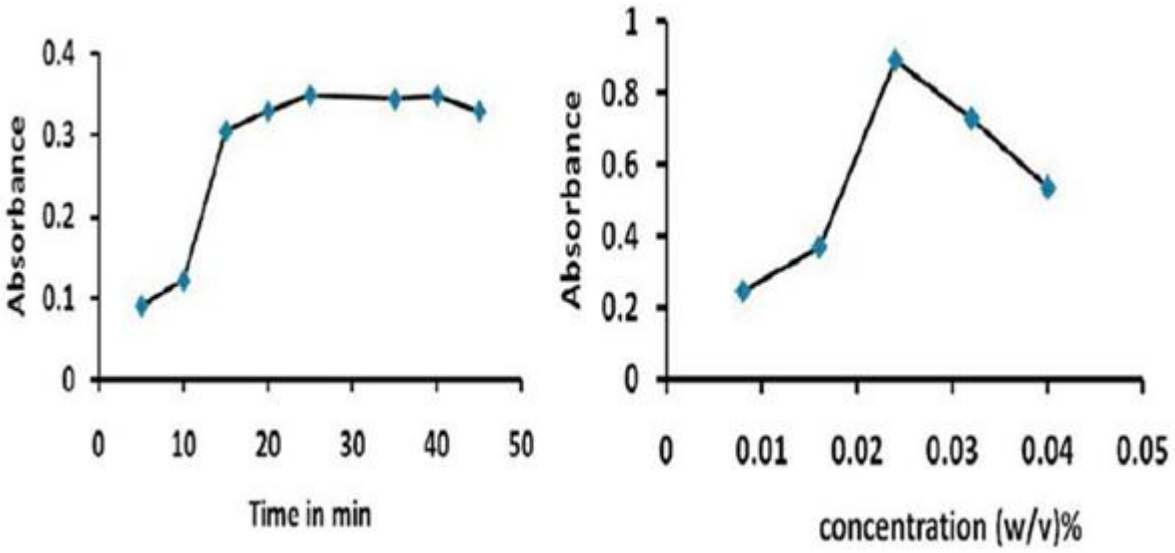


Figure 3. Impact of NBF-Cl concentrations on the L-tryptophan-NFB-Cl reaction. Effect of standing time on L-tryptophan's reactivity with NBF and L-tryptophan (20 µg/mL). 20 µg/mL of L-tryptophan

Product composition and validation of the suggested techniques

The composition of the product was ascertained by applying the equivalent mole method of continuous variation. As may be observed, Product I's L-tryptophan to NBF-Cl mole ratio is 1:1. The chemical pathway was assumed to continue as depicted in Scheme 2 based on the observed molar ratio [21]. In accordance with the International Conference on Harmonization's (ICH) recommendations, the methodologies' linearity, specificity, accuracy, repeatability, and precision were evaluated.

CONCLUSIONS

The spectrophotometric technique that was developed is sensitive, accurate, and exact. no interference from the additives and excipients that are commonly used. The method's applicability for analyzing the pharmaceuticals under study in both their pure and pharmaceutical formulation forms is demonstrated by statistical analysis.

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