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Spectrophotometric Estimation of L- 5-Hydroxytryptophan in *Griffonia simplicifolia* Extracts and Dosage Forms†

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Abstract: A simple, sensitive and precise spectrophotometric method has been developed for the determination of L-5-Hydroxytryptophan in *Griffonia simplicifolia* seed extracts and dosage forms. L-5-Hydroxytryptophan gives a blue colored chromogen with Folin-Ciocalteu reagent under alkaline conditions with absorption maxima at 736 nm. The chromogen obeys Beer's law in the concentration range 1-10 μg/ml. The proposed method is reproducible and statistically validated and recoveries range between 98.95% and 99.96%.

Keywords: Spectrophotometry; L-5-Hydroxytryptophan; Folin-Ciocalteu reagent; *Griffonia simplicifolia.*

1. Introduction

L-5-Hydroxytryptophan is an aromatic amino acid naturally produced by the human body from the essential amino acid, L-tryptophan obtained from dietary proteins. However, supplementation of L-tryptophan does not significantly increase L-5-Hydroxytryptophan levels in the human body. L-5-Hydroxytryptophan which is derived from seeds of *Griffonia simplicifolia Baill.* (Family: Caesalpinaceae) is recommended as a dietary supplement [1]. L-5-Hydroxytryptophan is a serotonin precursor and therapeutic administration of L-5-hydroxytryptophan has been shown to be effective in treating a wide variety of conditions, including depression, fibromyalgia, insomnia, obesity and chronic head aches [2].

Standardization of herbal products for ensuring the quality, therapeutic efficacy and safety assumes importance with the current increase in the demand for herbal products. Development of simple and inexpensive methods for the evaluation of herbal products helps the manufacturers of herbal products and regulatory authorities.

Various analytical techniques have been employed to estimate L-5-hydroxytryptophan by fluorescence spectrometry [3], liquid chromatography-mass spectrometry (LC-MS) [4], gas chromatography-mass spectrometry (GC-MS) [5]. Several high performance liquid chromatographic (HPLC) [6–12] analytical

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methods have been reported for the estimation of 5-hydroxytryptophan using various methods of detection and by capillary electrophoresis (CE) method [13].

In the present study, we report a simple and inexpensive spectrophotometric method for the estimation of L-5-hydroxytryptophan in the seed extracts of *Griffonia simplicifolia*. The method developed could be used in the routine estimation of L-5-hydroxytryptophan in the dosage forms.

2. Materials and methods

2.1. Reagents and instruments

L-5-Hydroxytryptophan mono hydrate was purchased from Sigma (USA). Sodium carbonate and Folin-Ciocalteu phenol reagent were obtained from Qualigens (Mumbai, India). Water purified by a Barnstead Nanopure system (Model: D3750, USA), to obtain nanopure water and it was used for all solutions. Aqueous 5 % (w/v) sodium carbonate solution and Folin-Ciocalteu phenol reagent diluted 1:5 with nanopure water were used. 50 mg of standard L-5-hydroxytryptophan was dissolved in 100 ml of nanopure water and diluted to get a working standard solution of 50 μg/ml with nanopure water. A Varian double beam UV-Visible spectrophotometer (model: Cary 50, Australia) with 1 cm matched quartz cuvettes was used for absorbance measurements.

2.2. Preparation of samples

Commercial samples of *Griffonia simplicifolia* seed extracts were obtained from M/s Laila Impex, Vijayawada, India and dosage forms purchased from USA.

2.2.1. *Griffonia simplicifolia* seed extracts

About 50 to 100 mg of *Griffonia simplicifolia* seed extract was taken and the sample solution was prepared as described for the standard solution and filtered, if insoluble material was present.

2.2.2. Dosage forms

To determine the content of L-5-hydroxytryptophan in capsules, the average weights were determined by weighing 20 capsules. Hard gelatin capsules were removed and the contents were finely powdered. The weight of the powder equivalent to 50 mg of L-5-hydroxytryptophan transferred in to a volumetric flask, nanopure water was added and sonicated for 10 minutes followed by diluting to 100 ml with nanopure water. The resultant solution was filtered on a whatman (no.1) filter paper.

2.3. Calibration curve

Aliquots (0.5–5.0 ml, 50 μg/ml) of standard L-5-hydroxytryptophan were transferred into a series of 25 ml volumetric flasks. To each volumetric flask, was added 10 ml of nanopure water, 2 ml of Folin-Ciocalteu reagent followed by 2 ml of sodium carbonate solutions were also added. The contents were mixed thoroughly and made up to the volume with nanopure water and kept at room temperature for 60 minutes. The absorbance values were measured at 736 nm against reagent blank.

2.4. Estimation procedure

To 1.0 ml of above prepared sample solutions were added 10 ml of nanopure water, 2 ml of Folin-Ciocalteu reagent followed by 2 ml of sodium carbonate solution. The contents were mixed thoroughly and made up to the volume 25 ml with nanopure water and kept at room temperature for 60 minutes. The absorbance values were measured at 736 nm against reagent blank. The amount of L-5-hydroxytryptophan was computed from the calibration graph.

2.5. Recovery study

To 1.0 ml of above prepared sample solution add 25 µg, 50 µg and 75 µg standard L-5-hydroxytryptophan separately. Add 10ml of nano pure water, 2 ml of Folin-Ciocalteu reagent followed by 2 ml of sodium carbonate solutions were added. The contents were mixed thoroughly and made up to 25 ml with nano pure water and kept at room temperature for 60 min. The absorbance values were measured at 736 nm against reagent blank.

3. Results and discussion

Preliminary experiments showed that L-5-hydroyxtryptophan gives an intense blue coloured chromogen with Folin-Ciocalteu reagent in presence of sodium carbonate. The blue coloured chromogen exhibited maximum absorbance at 736 nm (Figure1). The pH of the chromogen was in the range of 9.00 to 9.10. The optimum conditions for the proposed method were established by varying one parameter at a time and keeping the other parameters constant and observing the effect produced on the absorbance of the colored species [14]. The system attained maximum absorbance obtained after 60 minutes from the time of mixing of reagents. The intensity of the blue color is stable for three hours (Figure 2).

The method of analysis was validated for precision and accuracy. The other parameters like linearity range, molar absorptivity, Sandell's sensitivity and regression characteristics of the proposed method were presented in Table 1. The linearity was found over concentration range, 1-10 µg/ml, with a correlation coefficient of 0.9997. The linearity of calibration graph and the adherence of the system to Beer's law were validated by high value correlation coefficient.

The accuracy of the method was determined by performing the recovery study by adding known amount of L-5-hydroxytryptophan to the pre-analyzed samples ranging between 98.95 and 99.96%. The results were presented in Table 2.

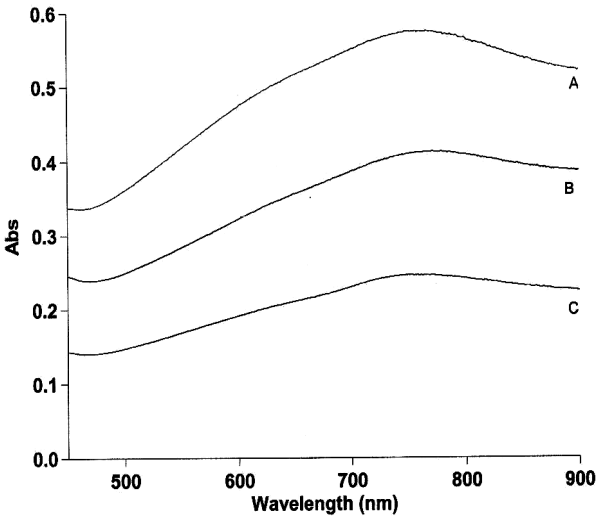

Figure 1. Absorption spectra of L-5-hydroxytryptophan- Folin-Ciocalteu system. A: standard L-5-hydroxytryptophan (5 µg/ml); B: *Griffonia simplicifolia* seed extract; C: dosage form

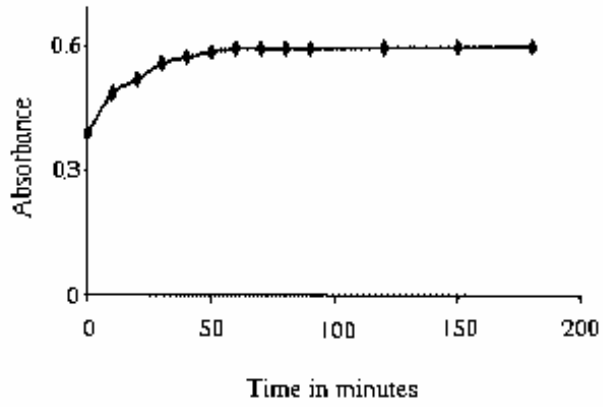

Figure 2. Stability of L-5-hydroxytryptophan-Folin-Ciocalteu system

Table 1. Method validation parameters

Parameter	Result
λ_{max} (nm)	736
Linearity range (μg/ml)	1 – 10
Molar absorptivity (L mol^{-1} cm^{-1})	25,000
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.07
Regression equation* Slope (a) Intercept (b) Correlation coefficient (r)	 0.109 1.40 x 10^{-2} 0.9997

* Y = ax+b, where x is the concentration of L-5-hydroxytryptophan in μg/ml and Y is the absorbance at 736 nm.

Table 2. Percent recovery studies (n=6)

Amount of L-5-hydroxytryptophan added (μg)	Amount of L-5-hydroxytryptophan recovered (μg)	Recovery (%)
25.0	24.7375	98.95± 0.308
50.0	49.8000	99.60± 0.089
75.0	74.9738	99.96± 0.052

L-5-Hydroxytryptophan effects the reduction of one of the oxygen atoms from tungstate or molybdate in Folin-Ciocalteu reagent, $3H_2O$, P_2O_5, $13WO_3.5MoO_3.10H_2O$ and $3H_2O$, P_2O_5, $14WO_3.4MoO_3.10H_2O$ [15], there by producing one or more of the possible species having characteristic intense blue colour.

The method was applied for the estimation of L-5-hydroxytryptophan in five different *Griffonia simplicifolia* seed extracts and three dosage forms. The results obtained were presented in Table 4. RSD (%) values were found to be in the range of 0.81 to 1.93%, which ascertains that the precision of the method is good.

Table 3. Interferencestudy (L-5-Hydroxytryptophan 25 μg)

Interfering substance	Amount of interfering substance added	L-5-hydroxytryptophan found (μg)
Starch	2.5 mg	25.00
Talc	2.5 mg	24.98
Gelatin	2.5 mg	25.00
Colloidal silicon dioxide	2.5 mg	25.00
Vitamin B_6	2.5 mg	24.90
Ascorbic acid	25 μg	36.00
Citric acid	25 μg	33.00

The interference studies in the estimation of L-5-hydroxytryptophan revealed that the excipients normally used and other additives like starch talc, gelatin, colloidal silicon dioxide and Vitamin B_6 used in capsules does not interfere even in 100 fold excess. However, presence of plant acids like ascorbic acid, citric acid and plant phenolics interferes in the estimation of L-5-hydroxytryptophan. The method affected by the presence of equal amounts of ascorbic acid or citric acid, where as in increasing the content of L-5-hydroxytryptophan (Table 3).

It is evident from Table 4, the results obtained by the proposed method almost is in good agreement with the results obtained from the HPLC method [12], which shows that *Griffonia simplicifolia* seed extracts does not contain any plant acids and plant phenolics. The lower assay value of capsule 3 is due to the low content of L-5-hydroxytryptophan only and the results are corroborated by the HPLC method [12].

However, the method may be used cautiously when the dosage forms are known to contain ingredients of interfering plant phenols and plant acids ascorbic acid and citric acid.

The method developed has an advantage of having highly intense coloured chromogen and specific to phenolic compounds, where as the other spectrophotometric methods which depends on UV absorption has interference from all other aromatic compounds and thus reduces the accuracy of the method.

The present method is simple, sensitive and has a good precision and accuracy and is useful for the estimation of L-5-hydroxytryptophan in either pure form, in *Griffonia simplicifolia* seed extracts or dosage forms.

Table 4. Estimation of L-5-hydroxytryptophan (5-HTP) by proposed method (n=6)

S.No	Sample	Labeled Amount (mg)	5-HTP by proposed method in (mg)	% RSD	5-HTP by HPLC method [12] (mg)
1.	*Griffonia simplicifolia* seed extracts [a]				
	Sample 1	-	94.50*	1.04	93.85 *
	Sample 2	-	95.86*	0.92	95.50 *
	Sample 3	-	96.26*	0.88	95.95 *
	Sample 4	-	95.03*	0.97	94.85 *
	Sample 5	-	22.68*	1.50	21.89 *
2.	Dosage forms[b]				
	Capsule 1	100.0	88.95	1.93	87.80
	Capsule 2	50.0	48.65	0.81	47.70
	Capsule 3	100.0	4.43	1.35	3.85

* Expressed as mg/100mg of extract [a]Commercial *Griffonia simplicifolia* seed extracts supplied by M/S Laila Impex, Vijayawada, India; [b]Capsule 1-Doctors Best 5-HTP (5-HTP – 100 mg; Other ingredients – Rice Powder, magnesium stearate, gelatin capsule), Capsule 2-Natrol 5-HTP (5-HTP – 50 mg; Other ingredients – Rice Powder, gelatin, silica, magnesium stearate), Capsule 3-The Vitamins Shoppe 5-HTP (5-HTP – 100 mg; Vitamin B_6 - 20 mg, Other ingredients – Rice powder, gelatin, water).

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