

## HPLC Estimation of 5-Hydroxytryptophan in *Griffonia simplicifolia* Extracts†

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A rapid and simple estimation procedure of 5-hydroxytryptophan (5-HTP) using high performance liquid chromatography (HPLC) has been developed for the estimation of 5-HTP in *Griffonia simplicifolia* seed extracts. HPLC separation was performed on a C18 column using 0.1% (v/v) phosphoric acid in water and acetonitrile (93 : 7) as mobile phase with detection at 275 nm. Four different commercially available *G. simplicifolia* extracts and two raw materials were analyzed to estimate the 5-hydroxytryptophan. The percentage of 5-hydroxytryptophan using the calibration curve was found to be  $94.75 \pm 0.038$ ,  $95.47 \pm 0.410$ ,  $94.02 \pm 0.037$  and  $22.55 \pm 0.394$  in extracts and  $9.93 \pm 0.002$  and  $12.09 \pm 0.004$  in raw materials.

**Key Words:** *Griffonia simplicifolia*, 5-Hydroxytryptophan (5-HTP), HPLC estimation.

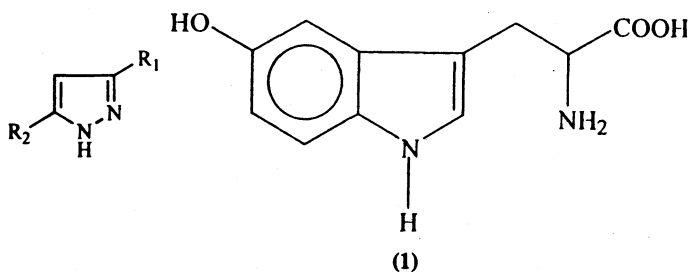
### INTRODUCTION

5-Hydroxytryptophan (5-HTP) (1) is an aromatic amino acid naturally produced by the body from the essential amino acid L-tryptophan. The biological significance of 5-HTP has been established<sup>1</sup>. It is an important metabolite in the biosynthesis of neurotransmitter serotonin. Serotonin appears to play a significant role in sleep, emotional moods, pain control, inflammation, intestinal peristalsis and other body functions<sup>2</sup>. 5-HTP is not present in significant amounts in a typical diet. The human body produces 5-HTP from L-tryptophan obtained from dietary proteins. However, eating food that contains L-tryptophan does not significantly increase 5-HTP levels. Supplement of 5-HTP which is derived from the seeds of *Griffonia simplicifolia* Baill. (Family Caesalpinaceae), a West African medicinal plant, is recommended<sup>3</sup>. Therapeutic administration of 5-HTP has been shown to be effective in treating a wide variety of conditions, including depression, fibromyalgia, insomnia, obesity and chronic headaches<sup>4</sup>. It is also known to be a potent antioxidant<sup>5</sup>.

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Various analytical techniques have been employed to estimate 5-HTP, including non-aqueous titration, fluorescence spectrometry<sup>6</sup>, liquid chromatography-mass spectrometry<sup>7</sup>, gas chromatography-mass spectrometry<sup>8</sup>. Several high performance liquid chromatographic analytical methods have been employed for estimation of 5-HTP using various kinds of detectors<sup>9-15</sup>. Most of the above cited HPLC methods are used for estimation of 5-HTP in biological materials, which are complex and need some prior separation of 5-hydroxytryptophan.

The present paper describes a rapid and simple estimation of 5-HTP in *Griffonia simplicifolia* extract and raw materials using high performance liquid chromatographic technique.



## EXPERIMENTAL

Commercial samples of *Griffonia simplicifolia* extract and seeds were obtained from M/s Laila Impex, Vijayawada, India. 5-Hydroxytryptophan monohydrate (Sigma Chemical Co., USA) and all solvents and chemicals used were of AR or HPLC grade.

The HPLC (Shimadzu) system used for estimation of 5-HTP was equipped with Phenomenex C18, 5 $\mu$  (250  $\times$  4.6 mm) column, LC-10AT pumps, SCL-10A system controller, SIL-10A auto injector, SPD-M10 AVP photodiode array detector set at wavelength of 275 nm for detection and class M10A software were used. A Millipore Swinnex type filter (pore size = 0.45  $\mu$ m) was used for filtration. Agilent 1100 series LC/MSD were used for obtaining mass spectral data.

**Mobile phase preparation:** 0.1% (v/v) phosphoric acid in water and acetonitrile (93 : 7) were mixed and filtered through 0.45  $\mu$ m membrane filter.

**Preparation of standard 5-HTP solution:** Standard 5-HTP solution 100  $\mu$ g/mL was prepared by dissolving suitable amount of 5-HTP monohydrate (Sigma) in mobile phase.

**Sample preparation of *G. simplicifolia* seed extract:** About 15 mg of test sample was weighed, dissolved in mobile phase and made up to 100 mL with mobile phase.

**Sample preparation of *G. simplicifolia* seed:** 1 g of *G. simplicifolia* seed (pulverized) was taken in a 250 mL round bottom flask. Extracted with 70% methanol (4  $\times$  25 mL) under reflux on water bath, each extraction for about 30 min to ensure the complete extraction of 5-HTP. All the extracts were combined, filtered and 5 mL of this extract was diluted to 25 mL with mobile phase.

**Chromatographic conditions:** The elution was carried out with isocratic system using 0.1% (v/v) phosphoric acid and acetonitrile (93 : 7) as mobile phase with a flow rate 1 mL/min at ambient temperature, run time about 15 min. The 5-HTP was quantified using class M10A software.

The linearity of the method was evaluated by analyzing a series of standard 5-HTP solutions. 20  $\mu$ L of each of the six working standard solutions was injected into the HPLC. The elutions was carried out as described above. Standard calibration curve was obtained by plotting the concentration of standard 5-HTP vs. peak area (average of three runs).

The calibration range was chosen to reflect normal 5-HTP in *G. simplicifolia* extract samples. This range included concentration from the lower limit of quantification to the upper limit of quantification. The limit of quantification (LOQ) was defined as the lowest standard 5-HTP concentration which can be determined with an accuracy and precision of less than 2%.

**Determination of 5-HTP:** The sample volume was 20  $\mu$ L. 5-HTP concentration was calculated on the basis of linear calibration function and with regard to the dilution factor. The content of 5-HTP was expressed as grams per 100 grams of extracts or plant materials.

## RESULTS AND DISCUSSION

HPLC analysis of 5-HTP showed single peak at retention time  $6.1 \pm 0.45$  min. The typical chromatogram at 275 nm has been shown in Fig. 1. Calibration graph

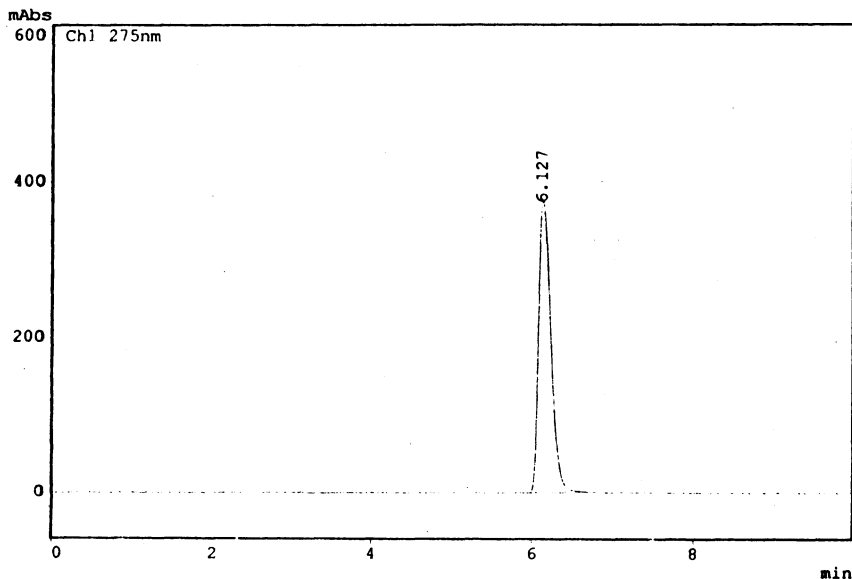


Fig. 1. Typical chromatogram of 5-hydroxytryptophan in *Griffonia simplicifolia* extract

was prepared to determine the 5-HTP content of different *Griffonia* samples. Calibration curve was derived from three independent injections of six concentrations of 5-HTP vs. the peak areas. Linearity was found in the concentration range between 0.5 and 5  $\mu$ g of 5-HTP with high reproducibility and accuracy (Fig. 2). The regression analysis of the experimental data shows the linear relationship with

excellent correlation coefficient of 0.9999. Accuracy of the method verified by the recovery studies listed in Table-1. Using this method, 5-HTP in different grades of *G. simplicifolia* seed extract and *G. simplicifolia* seeds have been estimated. The results are listed in Table-2. All the samples were analyzed with no interference from other compounds. The efficiency of the column is 6000 theoretical plates: tailing factor of the analyte 5-HTP is < 1.7. The identity was confirmed by determination of retention time and by spiking with standard and also confirmed with mass spectrometry.

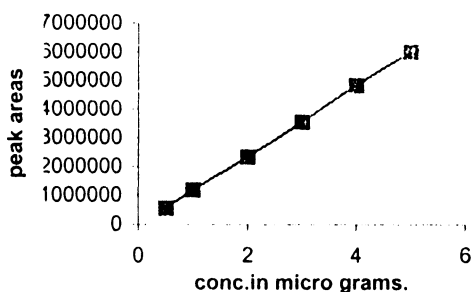


Fig. 2. Linear relationship between peak area response and concentration of 5-hydroxytryptophan.

TABLE-1  
RECOVERY STUDY

S.No.	Amount of 5-HTP added (mg)	Amount of 5-HTP recovered (mg)	% Recovery*
1.	0.7500	0.7495	99.93 ± 0.094
2.	1.5000	1.5030	100.20 ± 0.118
3.	2.2500	2.2501	100.00 ± 0.112

\*Average of three determinations

TABLE-2  
PERCENTAGE (w/w) OF 5-HTP IN *GRIFFONIA SIMPLICIFOLIA* EXTRACTS AND SEEDS BY PROPOSED HPLC METHOD

S. No.	Name of the sample	% 5-HTP*
	<i>Griffonia simplicifolia</i> extract	
1	Sample 1	94.75 ± 0.038
	Sample 2	95.47 ± 0.410
	Sample 3	94.02 ± 0.037
	Sample 4	22.55 ± 0.394
	<i>Griffonia simplicifolia</i> seed	
2	Sample 1	9.93 ± 0.002
	Sample 2	12.09 ± 0.004

\*Average of six determinations

Preliminary experiments indicate that the chromatographic conditions described are applicable to the determination of 5-HTP in *G. simplicifolia* extracts and raw materials. The method described is simple, requiring minimal sample handling and preparation.

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