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Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0052 - Griffonia for 5-HTP by HPLC

Botanical Name: *Griffonia simplicifolia*

Common Names: 5-HTP

Parts of Plant Used: Seeds

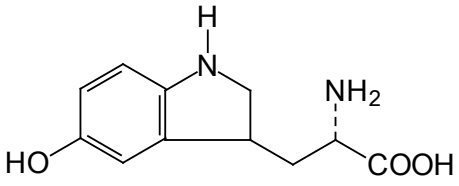
Uses: As an antidepressant, to treat serotonin deficiency syndrome, to treat headache, to control weight.

Modes of Action:

L-5-Hydroxytryptophan (5-HTP), a serotonin precursor, is the bioactive component in griffonia seeds. Several double-blind placebo-controlled clinical trials have been performed on this compound. 5-HTP was found to treat headache, to work as an antidepressant drug, to reduce body weight, to treat primary fibromyalgia syndrome, and to inhibit panic.[1–7]

Chemical Markers:

L-5-hydroxytryptophan is the main chemical component in griffonia seeds and a recent study found that the content of 5-HTP can reach 20.83% in the fresh material.[8] Indole-3-acetylaspartic acid, 5'-hydroxyindole-3-acetic acid, griffonin, and griffonilide also were detected in griffonia.[9,10] 5-Hydroxytryptamine was found in the pods and, in lower concentration, in the leaves of mature plants. In griffonia seed oil, the fatty acid composition is 18:2 = 60%, 16:0, 18:0, and 18:1 = 9% to 18%, and 20:0 = 3% to 4%. The main sterols are ß-sitosterol (60%), stigmasterol (29%), and campesterol (11%).[11] As 5-HTP has been proven to be the bioactive component, it is used as the marker compound for quality control of griffonia seed extract.



L-5-Hydroxytryptophan

Methods of Analysis

HPLC is the most accepted method for 5-HTP analysis. Various solvents have been used to extract 5-HTP from griffonia seeds; 50% methanol proved to be the most effective solvent.[8]

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Method 1:

The method of Lemarie and Adosraku[8] was used.

Sample Preparation:

Transfer 1 g of powdered seed sample to a 100-mL volumetric flask, add 70 mL of 50% methanol, and shake vigorously for 10 minutes. Fill to volume with 50% methanol.

Chromatography:

Column: Tosahaas ODS-80TS TSK-GEL9R0, 5 µm, 250 × 4.6 mm.
Mobile phase: Water (5 mM phosphate buffer at pH 4.8)–methanol (97:3) isocratic.
Flow rate: 1.5 mL/minute
Injection volume: 20 µL
Detection wavelength: 275 nm

Validation Data:

Not available

Method 2:

The unpublished method of Mingfu Wang was used.

Sample Preparation:

Acurately weigh 20 mg of 5-HTP or 100 mg of griffonia seed powder into a 100-mL volumetric flask, add 75 mL of 50% methanol and sonicate for 30 minutes. Cool to room temperature and fill to volume with 50% methanol.

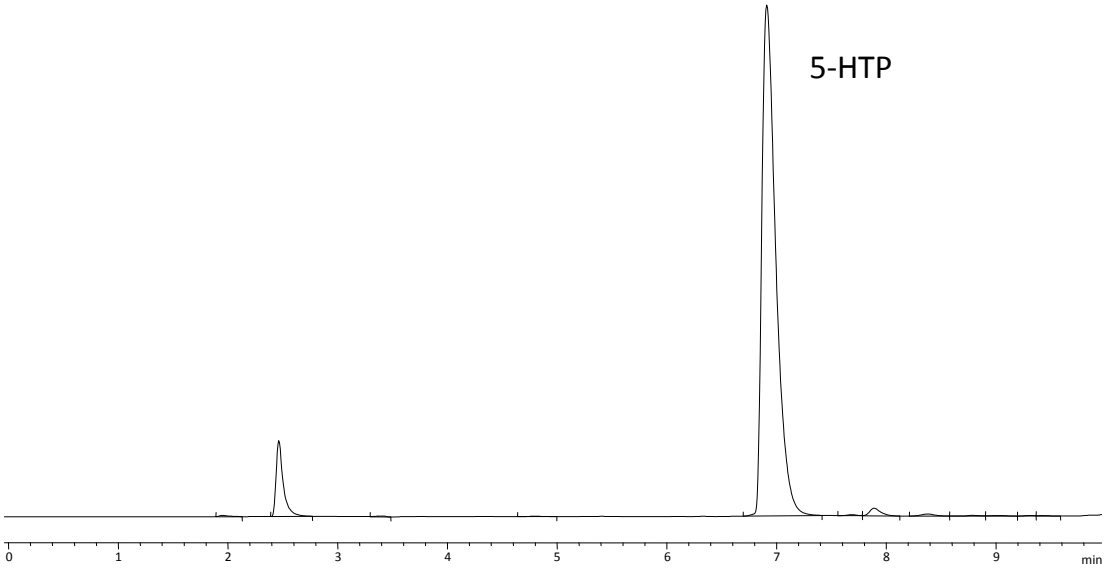
Chromatography:

Column: Phenomenex Luna C18 (2), 5 m, 250 × 4.6 mm.
Mobile phase: Water (0.1% trifluoroacetic acid)–acetonitrile.
Gradient: 2%B to 16%B in 10 minutes.
Flow rate: 0.8 mL/minute
Injection volume: 10 µL
Detection wavelength: 280 nm

Validation Data:

Not available

Representative HPLC Chromatogram for Griffonia Seed Powder Run by Method 2.



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