

Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-May-2014
Document Type: Dietary Supplement Monographs
DocId: GUID-7B37AFC1-E29C-4147-B629-E438DFE4487D_1_en-US
DOI: https://doi.org/10.31003/USPNF_M18968_01_01
DOI Ref: rup5l

© 2025 USPC
Do not distribute

Red Clover Tablets

DEFINITION
Red Clover Tablets contain Powdered Red Clover Extract. Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of Powdered Extract, calculated as isoflavones.

IDENTIFICATION

• **A. HPLC IDENTIFICATION TEST**

Analysis: Proceed as directed for the *Content of Isoflavones*.
Using the values obtained in the test for *Content of Isoflavones*, calculate the ratio of 5,7-dihydroxyisoflavones to 7-hydroxyisoflavones:

$$\text{Result} = (B + G)/(D + F)$$

- B* = percentage of biochanin A
- G* = percentage of genistein
- D* = percentage of daidzein
- F* = percentage of formononetin

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks for daidzein, genistein, formononetin, and biochanin A at retention times that correspond to those in the chromatogram of *Standard solution A*, and the ratio of 5,7-dihydroxyisoflavones to 7-hydroxyisoflavones is between 0.1 and 10.

STRENGTH

• **CONTENT OF ISOFLAVONES**

Solution A: Acetonitrile and water (1:3) containing 0.05% trifluoroacetic acid
Solution B: Acetonitrile containing 0.05% trifluoroacetic acid
Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
2.5	87	13
7.5	80	20
7.8	73	27
8.0	55	45
11.0	50	50
13.0	40	60

Time (min)	Solution A (%)	Solution B (%)
15.0	26	74
16.0	0	100
18.1	100	0
23.0	100	0

Solvent: Alcohol and water (1:1)

Standard stock solution: Transfer a quantity of [USP Powdered Red Clover Extract RS](#), equivalent to 30 mg of the labeled content of isoflavones, to a 250-mL volumetric flask. Add 15 mL of dehydrated alcohol, sonicate until dissolved, and dilute with *Solvent* to volume.

Standard solution A: Evaporate 50 mL of the *Standard stock solution* to dryness under vacuum. Add 15 mL of 2 N hydrochloric acid, and heat in a water bath for 30 min. Quantitatively transfer the resulting solution, with the aid of 15 mL of alcohol, to a 50-mL volumetric flask, and dilute with *Solvent* to volume. Centrifuge, or filter through a membrane of 0.45-µm or finer pore size.

Standard solution B: 0.1 mg/mL of [USP Formononetin RS](#), 0.02 mg/mL of [USP Genistein RS](#), 0.02 mg/mL of [USP Daidzein RS](#), and 0.1 mg/mL of [USP Biochanin A RS](#) in a mixture of *n*-propanol and water (1:1). Sonicate, and filter through a membrane of 0.45-µm or finer pore size.

Sample stock solution: Weigh NLT 20 Tablets, and pulverize. Transfer the equivalent of 40 mg of the labeled amount of isoflavones to a 250-mL volumetric flask. Add 15 mL of water, shake to disperse the powder, add 15 mL of dehydrated alcohol and 200 mL of *Solvent*, and sonicate for 30 min. If dark particles are present in the bottom of the flask, sonicate again for an additional 10 min or until they disappear. Cool to room temperature, dilute with *Solvent* to volume, and filter.

Sample solution: Transfer 50.0 mL of the resulting solution to a round-bottom flask, and evaporate to dryness under vacuum. Add 15 mL of 2 N hydrochloric acid, and heat in a water bath for 30 min. Quantitatively transfer this solution, with the aid of 15 mL of alcohol, to a 50-mL volumetric flask, and dilute with *Solvent* to volume. Pass 5 mL of the solution through a filter of 0.45-µm pore size, discarding the first 4 mL of the filtrate. Collect the remaining 1 mL of filtrate for testing.

Chromatographic system

(See [Chromatography \(621\). System Suitability.](#))

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; end-capped 5-µm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution A* is similar to the reference chromatogram provided with the lot of [USP Powdered Red Clover Extract RS](#) being used.

Tailing factor: NMT 2.0 for the formononetin peak, *Standard solution B*

Relative standard deviation: NMT 2.0%, determined from the formononetin peak in repeated injections, *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the peaks corresponding to daidzein, genistein, formononetin, and biochanin A in the *Sample solution* chromatogram by comparison with the chromatogram obtained from *Standard solution A* and the reference chromatogram. Measure the areas of the analyte peaks.

Calculate the content of each isoflavone, in mg, in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D$$

r_U = peak response for each relevant isoflavone from the *Sample solution*

r_S = peak response for daidzein, genistein, formononetin, or biochanin A from *Standard solution B*

C_S = concentration of daidzein, genistein, formononetin, or biochanin A in *Standard solution B* (mg/mL)