USP Monographs in a Small Contract Laboratory: Case Studies

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GAAS Analytical
Tucson, Arizona
Turmeric/Curcuminoids
Dietary Supplements

Supplement Facts
Serving Size - 1 Softgel
Servings Per Container - 60

<table>
<thead>
<tr>
<th>Amount per Serving</th>
<th>% Daily Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin Extract</td>
<td>250 mg</td>
</tr>
<tr>
<td>(as 95% curcuminoids)</td>
<td>†</td>
</tr>
</tbody>
</table>

† Daily Value not established.

Other Ingredients: Gelatin, Extra Virgin Olive Oil, Medium Chain Triglycerides, Glycerin, Purified Water, Yellow Beeswax, Soy Lecithin.

Suggested Use: As a dietary supplement, take 1 – 2 softgels daily. Or use as directed by your healthcare professional.

*These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

Nutrition Facts
Serving Size 3 Capsules
Servings Per Container 30

<table>
<thead>
<tr>
<th>Amount Per Serving</th>
<th>% Daily Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine Sulfate</td>
<td>1500 mg</td>
</tr>
<tr>
<td>White Willow Bark Extract</td>
<td>250mg</td>
</tr>
<tr>
<td>MSM (Methysulfonylmethane)</td>
<td>500mg</td>
</tr>
<tr>
<td>Hyaluronic Acid</td>
<td>4mg</td>
</tr>
<tr>
<td>Cayenne 40m H.U.</td>
<td>50mg</td>
</tr>
<tr>
<td>Ginger Root Extract 4:1</td>
<td>250mg</td>
</tr>
<tr>
<td>Boswellia Serrata Extract</td>
<td>125mg</td>
</tr>
<tr>
<td>Turmeric Extract 25:1</td>
<td>50mg</td>
</tr>
</tbody>
</table>

Proprietary Blends
- Boswellia Serrata Extract standardized to 65% boswellic acid
- Turmeric Extract 25:1 standardized to 95% curcuminoids

*percent Daily Values are based on a 2,000 calorie diet.

Ingredients: Rice Flour, Gelatin, Vegetable Magnesium Stearate And Silicon Dioxide. CONTAINS: Crustacean Shellfish (Shrimp, Crab).
USP Monograph Search

Results for "curcumin extract" (6 results)

Document Results

**Powdered Turmeric Extract**
- Monographs: Official as of 1-May-2020
- Powdered Turmeric Extract: Powdered Turmeric Extract is prepared from the pulverized rhizomes.

**Curcuminoids**
- Monographs: Official as of 1-May-2020
- of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in the portion of Curcuminoids taken. Result = (FU

**Powdered Turmeric**
- Monographs: Official as of 1-May-2017
- of Curcuminoids. Analysis Samples: Standard solution B and Sample solution Calculate the percentages of curcumin

**Turmeric**
- Monographs: Official as of 1-May-2017
- of Curcuminoids. Analysis Samples: Standard solution B and Sample solution Calculate the percentages of curcumin

**Powdered Turmeric Extract**

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USP Monograph
Curcuminoids

DEFINITION
Curcuminoids is a partially purified natural complex of diarylheptanoid derivatives isolated from Turmeric, Curcuma longa L. It contains NLT 98.0% of curcuminoids, calculated on the dried basis, as the sum of curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. It contains NLT 70.0% and NMT 80.0% of curcumin, NLT 15.0% and NMT 25.0% of desmethoxycurcumin, and NLT 2.5% and NMT 6.5% of bisdesmethoxycurcumin.

B. HPLC
Analysis: Proceed as directed in the test for Content of Curcuminoids.
Acceptance criteria: The retention times of the peaks for curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin of the Sample solution correspond to those of Standard solution A and Standard solution B.

COMPOSITION
• CONTENT OF CURCUMINOIDS
Mobile phase: Tetrahydrofuran and 1 mg/ml of citric acid in water (4:6)
Standard solution A: 40 μg/ml of USP Curcuminoids RS in Mobile phase.
Standard solution B: A composite solution containing 40 μg/ml of USP Curcumin RS, 10 μg/ml of USP Desmethoxycurcumin RS, and 2.0 μg/ml of USP Bisdesmethoxycurcumin RS in Mobile phase. Use sonication if necessary. Before injection, pass through a filter of 0.45-μm pore size, and discard the initial 10 ml of the filtrate.
Sample stock solution: Transfer about 20 mg of Curcuminoids, accurately weighed, to a 50-mL volumetric flask, add 30 mL of acetone, and sonicate for 30 min. Dilute with acetone to volume, mix, and centrifuge.
Sample solution: Transfer 5.0 mL of the Sample stock solution to a 50-mL volumetric flask. Dilute with Mobile phase to volume, and mix. Before injection, pass through a filter of 0.45-μm pore size, and discard the initial 10 mL of the filtrate.
Chromatographic system
See Chromatography (521), System Suitability.
Mode: LC
Detector: Vis 420 nm
Column: 4.6-mm × 25-cm, 5-μm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 μL
System suitability
Samples: Standard solution A and Standard solution B
(Note-The relative retention times for the curcumin, desmethoxycurcumin, and bisdemethoxycurcumin peaks are 1.0, 1.2, and 1.4, respectively)
Suitability requirements
Chromatogram similarity: The chromatogram of Standard solution A is similar to the reference chromatogram provided with USP Curcuminoids RS.
Resolution: NLT 2.0 between curcumin and desmethoxycurcumin peaks and desmethoxycurcumin and bisdesmethoxycurcumin peaks, Standard solution B
Tailing factor: NMT 1.5 for bisdesmethoxycurcumin, desmethoxycurcumin, and curcumin peaks, Standard solution B
Relative standard deviation: NMT 2.0% for the desmethoxycurcumin peak, in replicate injections, Standard solution B
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Delayed-release (enteric-coated) soft shell capsules: Place 1 softgel capsule in each of the six tubes of the basket. Omit the use of a disk. Operate the apparatus using simulated gastric fluid TS, maintained at 37 ± 2°C, as the immersion fluid. After 1 h of operation in simulated gastric fluid TS, lift the basket from the fluid and observe the softgels: the softgels show no evidence of disintegration or rupture that would permit the escape of the contents. Operate the apparatus with disks using simulated intestinal fluid TS, maintained at 37 ± 2°C, as the immersion fluid for NMT 60 min. Lift the basket from the fluid and observe the capsules.
Optimization of the Extraction Method – Extraction solvent

![Graph showing area under the curve for different solvents and compounds.]

- Methanol
- 80:20 Methanol:Water
- 50:50 Methanol:Water
- 20:80 Methanol:Water
- Acetonitrile

Area under the curve (mAU*s)

Bis-demethoxycurcumin
Demethoxycurcumin
Curcumin
Extraction time and method

![Graph showing area under the curve (mAU*s) for different extraction methods and compounds.](image)
Optimization of the Extraction Method – Extraction Efficiency

AUC: 88.1

AUC: 42.9
Optimization of the Extraction Method – Extraction Efficiency

![Graph showing the area under the curve for bis-demethoxycurcumin, demethoxycurcumin, and curcumin with different extraction methods.](image)

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Chromatographic System

USP method

Chromatographic system
(See Chromatography (621), System Suitability.)

Mode: LC
Detector: Vis 420 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 20 µL

In-house availability

Phenomenex
Kinetex Biphenyl
4.6 x 150 cm, 2.6 µm

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Method Translator - Agilent

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## Method Translator - ThermoFisher

**LC Method Transfer Calculator**

Use at your own risk. Prepopulated data are estimates only. Thermo Fisher Scientific takes no responsibility for the accuracy of data, calculations or results.

<table>
<thead>
<tr>
<th>Current Column</th>
<th>Planned Column</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length (mm):</strong></td>
<td><strong>Length (mm):</strong></td>
</tr>
<tr>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td><strong>Diameter (mm):</strong></td>
<td><strong>Diameter (mm):</strong></td>
</tr>
<tr>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td><strong>Particle Size (µm):</strong></td>
<td><strong>Particle Size (µm):</strong></td>
</tr>
<tr>
<td>5.0</td>
<td>2.6</td>
</tr>
</tbody>
</table>

**Peak Details (Critical Pair):**

<table>
<thead>
<tr>
<th>Actual R&lt;sub&gt;g&lt;/sub&gt; (Resolution Factor):</th>
<th>Predicted R&lt;sub&gt;g&lt;/sub&gt; Change Factor:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>1.07 (7.4%)</td>
</tr>
</tbody>
</table>

**Predicted R<sub>g</sub>:**

2.15 Baseline resolution achieved
**Method Translator - ThermoFisher**

**Current Method Conditions**

- **Flow (mL/min):** 1.000
- **Injection Volume (µL):** 120
- **Max Observed Pressure:** 1.923
- **Pressure Units:** bar
- **Number of samples:** 1
- **Data Collection Rate (Hz):** 35.00

**Recommended Method Conditions**

- **Boost Factor:** 1.00
- **Adjust Flow:**
  - **Flow (mL/min):** 120
  - **Injection Volume (µL):** 120
  - **Estimated Max Observed Pressure:** 512 bar
- **Number of samples:** 1
- **Data Collection Rate (Hz):** 35.00

**Current Gradient Table**

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
<th>%C</th>
<th>%D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>60.0</td>
<td>40.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>60.00</td>
<td>60.0</td>
<td>40.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Planned Gradient Table**

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
<th>%C</th>
<th>%D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>60.0</td>
<td>40.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>18.72</td>
<td>60.0</td>
<td>40.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
USP Reference Standard (Curcuminoids)

USP Reference Standard
CURCUMINOID 300 mg

[Typical Chromatogram]

USP Curcuminoids RS
Catalog Number: 1151866
Lot: RO190
Monograph: Curcuminoids
Publication: USP41/NF36
Test: Content of Curcuminoids
Sample: Standard Solution A

Curcumin
Demethoxycurcumin
Bisdemethoxycurcumin

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Demethoxy-curcumin and bis-demethoxy-curcumin reference standards are also available.
Case Study #2 – Quantitation of Curcuminoids and Gingerols

Nutrition Facts
Serving Size 3 Capsules
Servings Per Container 30

Amount Per Serving

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Serving Size</th>
<th>%DV</th>
</tr>
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<tbody>
<tr>
<td>Glucosamine Sulfate</td>
<td>1500mg</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
<td>MSM</td>
<td>500mg</td>
<td>-</td>
</tr>
<tr>
<td>(Methylsulfonylmethane)</td>
<td>4mg</td>
<td>-</td>
</tr>
<tr>
<td>Hyaluronic Acid</td>
<td>50mg</td>
<td>-</td>
</tr>
<tr>
<td>Cayenne 40m H.U.</td>
<td>250mg</td>
<td>-</td>
</tr>
<tr>
<td>Ginger Root Extract 4:1</td>
<td>125mg</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Turmeric Extract 25:1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Proprietary Blends

- Boswellia Serrata Extract
  standardized to 65% boswellic acid

- Turmeric Extract 25:1
  standardized to 95% curcuminoids

"percent Daily Values are based on a 2,000 calorie diet.

Ingredients: Rice Flour, Gelatin, Vegetable Magnesium Stearate And Silicon Dioxide. CONTAINS: Crustacean Shellfish (Shrimp, Crab).
Ginger Capsules

DEFINITION
Ginger Capsules are prepared from powdered ginger and contain NLT 90.0% and NMT 110.0% of the labeled amount of gingerols, gingerdiones, and shogaols, and NLT 90.0% of the labeled amount of volatile oils.

IDENTIFICATION
- A.
  Analyze an amount of the contents of Capsules equivalent to 5 g of ginger. To an amount equivalent to 1 g of ginger add 3 mL of glacial acetic acid, and prepare by dividing 1 part of glacial acetic acid with 1 part of water, and shake for 15 min. Filter, and add a few drops of ammonium solution to the filtrate. Acceptance criteria: NMT a slight turbidity is produced.
- B.
  Sample: (see Articles of Botanical Origin (131), Alcohol-Soluble Extractions (Method 2)). Collect the filtrate in a 100 mL volumetric flask, and dilute with alcohol to volume. Evaporate 15 mL of the filtrate to 10 mL of alcohol, cool, add 20 mL of water, and NLT 0.2 mg/mL of USP Ginger Constituent Mixture RS, and filter.

CHROMATOGRAPHIC ANALYSIS
(See Chromatography (621), System Suitability)
Model: HPLC
Detector: UV 282 nm
Column: 4.6 mm × 25 cm, packing L1
Flow rate: 1 mL/min
Injection size: 50 μL
System suitability
Samples: Standard USP Ginger Constituent Mixture RS, USP Ginger Capsules RS, USP Capsicum Annuum Capsules RS

Relative standard deviation: NMT 2.5% for the capsaicin peak for replicate injections, Standard

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>29</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Standard solution: 0.1 mg/mL of USP Ginger Constituent RS in methanol

Sample solution: Mix and finely powder the contents of NLT 20 Capsules, and transfer an amount equivalent to 2.0 g of powdered ginger to a glass-stoppered conical flask. Add 50 mL of alcohol, invert a stopper into the flask, and macerate for 24 h, shaking frequently during the first 8 h, and then allowing to stand for 16 h. Filter, and use the filtrate.
Wavelength Selection

6-Gingerol

Curcumin

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Peak Purity/Resolution

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Thank you!

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