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Application Note

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

0067 - Turmeric for Curcuminoids by HPLC

Botanical Name: *Curcuma longa L.; Curcuma domestica Val.*

Common Names: Common turmeric, haridra, Indian saffron, Jiang-Huang, yellow ginger

Parts of Plant Used: Rhizomes

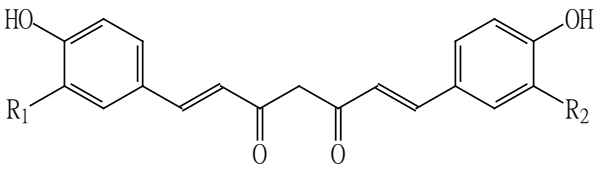
Uses: As a food colorant, as an anticancer agent, as an anti-inflammatory agent.

Modes of Action:

The health benefits of turmeric have been supported by research and the bioactivities of curcumins have been studied. Curcumins were found to be antioxidant and anti-inflammatory agents and to suppress tumor initiation, promotion, and metastasis. Curcumins suppress proliferation of a wide variety of tumor cells: they down-regulate transcription factors AP-1, Egr-1, and NF-κB; down-regulate the expression of COX2, LOX, NOS, MMP-9, TNF, uPA, chemokines, cell surface adhesion mols, and cyclin D1; down-regulate growth factor receptors; and inhibit the activity of c-Jun N-terminal kinase, protein tyrosine kinases, and protein serine/threonine kinases.[1,2]

Chemical Markers:

Three major curcuminoids (diarylheptane derivatives) are found in turmeric rhizomes: curcumin (the major one) and methoxycurcumin and bisdemethoxycurcumin. The essential oil of turmeric roots has been studied by GC and GC–MS: the rhizome essential oils were highly rich in α- and ß-turmerones (40.8%), myrcene (12.6%), 1,8-cineole (7.7%), and p-cymene (3.8%).[3]



Curcumin: $R_1 = OCH_3$, $R_2 = OCH_3$
Demethoxycurcumin: $R_1 = OCH_3$, $R_2 = H$
Bisdemethoxycurcumin: $R_1 = H$, $R_2 = H$

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Methods of Analysis

The curcuminoid content in tumeric powders or powder extracts has been analyzed by various methods including spectrophotometry, TLC, GC–MS, LC–MS, and capillary electrophoresis, but HPLC is the most widely used analytical method.[4–7]

Extraction is key for accurate analysis of cucurmins in tumeric. Generally, pure methanol is the selected solvent. The extraction should be handled carefully, avoiding use of strong acids and alkaloids in the extraction and avoiding extra metals in the extraction solvent. The extraction solution should be analyzed as soon as possible because curcumins may degrade.[8]

Method 1:

The HPLC method of Jayaprakasha et al.[4] was used.

Sample Preparation:

Extract turmeric powder (1 g) with 50 mL of hexane using a Sohlet extractor for 30 minutes. Discard the hexane and extract the sample with 50 mL of methanol for 2 hours.

Chromatography:

Column: Waters µBondapak C18, 300 × 4.6 mm.
Mobile phase: Solvent A = water with 2% acetic acid, solvent B = acetonitrile, solvent C = methanol.
Gradient:

Time (minutes)	%A	%B	%C
0	50	45	5
15	30	65	5
20	50	45	5

Flow rate: 1.0 mL/minute
Injection volume: 10 µL
Detection wavelength: 425 nm

Validation Data:

Linearity: 0.0625 to 2.9 mcg for curcumin, demethoxycurcumin, and bisdemethoxycurcumin with correlation coefficients over 0.9844.
Accuracy: Not specified
Precision: Not specified
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: LOQ = 0.05 mcg.

Method 2:

The method of Hiserodt et al.[6] was used.

Sample Preparation:

Sonicate 10 mg of sample in 10 mL of methanol.

Chromatography:

Column: Supelcosil LC18, 5 µm, 250 × 4.6 mm.
Mobile phase: Solvent A = water with 1% citric acid (pH adjusted to 3 with sodium hydroxide,) solvent B = acetonitrile.
Gradient:

Time (minutes)	%A	%B
0	50	50
10	50	50
40	20	80

Injection volume: 10 µL
Flow rate: 1 mL/minute
Detection wavelength: 423 nm

Validation Data:

Linearity: 186 to 929 pg for curcumin, 52 to 258 pg for demethoxycurcumin, and 14 to 71 pg for bisdemethoxycurcumin with correlation coefficients over 0.9989.
Accuracy: Not specified
Precision: 1.68% RSD for curcumin, 0.60% RSD for demethoxycurcumin, and 0.49% RSD for bisdemethoxycurcumin.
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: LOQ = 0.05 mcg.

Method 3:

The unpublished method of Mingfu Wang was used.

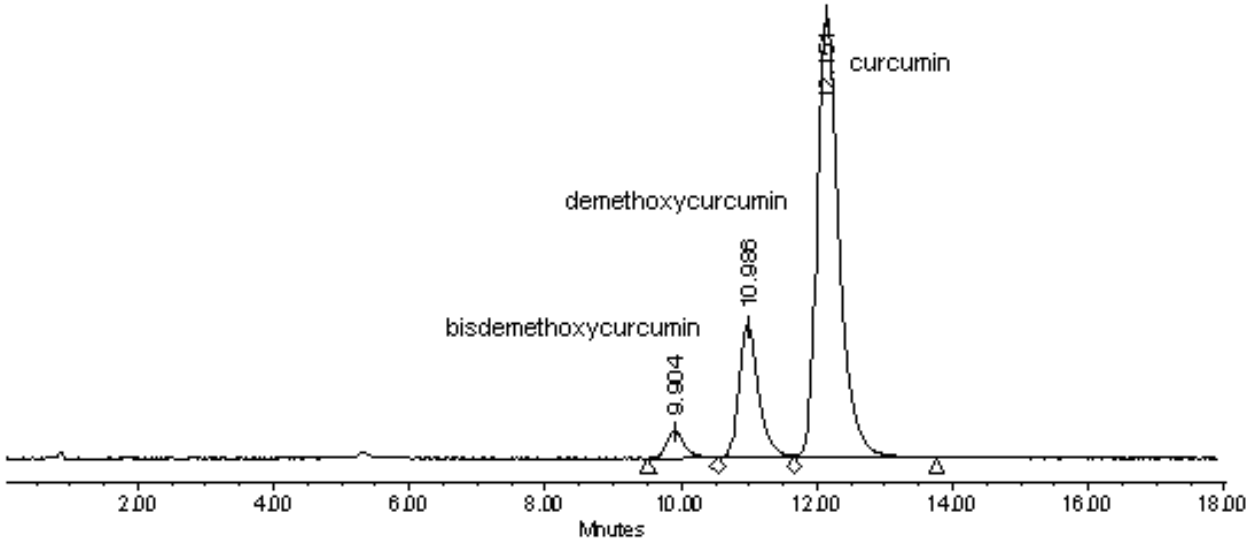
Sample Preparation:

Sonicate 500 mg of tumeric powder or 30 mg of curcumin extract in 70 mL of methanol for 30 minutes. Cool to room temperature, and fill to 100 mL with methanol for HPLC analysis.

Chromatography:

Column: Phenomenex Prodigy ODS 3, 100Å, 5 µm, 150 × 320 mm.
Mobile phase: Solvent A = water with 0.2% phosphoric acid, solvent B = acetonitrile, A/B (60:40) isocratic.
Injection volume: 10 µL
Flow rate: 1 mL/minute
Detection wavelength: 430 nm

Representative HPLC Chromatogram of a Turmeric Extract Run by Method 3



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