

What's in Your Supplement?

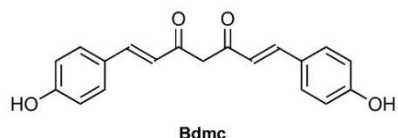
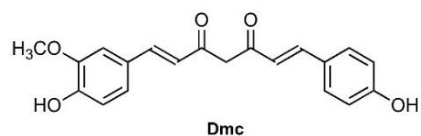
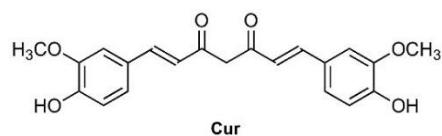
Summary

This application note explores how the claims made on the labels of dietary supplements can differ from the contained ingredients through deformation of a curcumin nutraceutical using liquid chromatography-mass spectrometry (LC-MS).

Background



Turmeric (*Curcuma longa*) is a member of the ginger family, easily recognized by its vivid orange color. Turmeric possesses anti-inflammatory and anti-oxidant capabilities that stem from three constituent curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) (Figure 1). As a result, turmeric curcumin has been promoted as a natural method to treat, or supplement treatment of, a number of chronic conditions, including neurodegenerative, cardiovascular, pulmonary, and autoimmune diseases, as well as some types of cancer. Despite curcumin's potential as a therapeutic agent, clinical trials have been limited due to a lack of oral bioavailability and rapid plasma clearance.



Although the latter properties prevent pharmaceutical companies from utilizing curcumin, industries that produce and distribute dietary supplements are not subject to the same FDA regulations, and FDA approval is not required before producing or selling. More specifically, there are currently no requirements that supplements be proven safe prior to marketing and claims made on labels do not need to be accurate or truthful to the FDA's satisfaction. As a result, some marketed supplements contain less, or sometimes none, of the active ingredient they purport to contain. Here, we use liquid chromatography-mass spectrometry (LC-MS) to evaluate the curcuminoid content of a curcumin supplement relative to the claims made on the label.

Figure 1: The primary curcuminoids found within *Curcuma longa*: curcumin (Cur), demethoxycurcumin (Dmc), and bisdemethoxycurcumin (Bdmc).

Samples

Samples were obtained from a bottle of commercial curcumin supplement (Figure 2). The manufacturer's label indicated that each capsule contained 180 mg of curcumin. For comparison, curcumin from *Curcuma longa* was purchased from Sigma Aldrich.

Supplement Facts		
Serving Size 2 capsules		
Amount per 2 capsules		%DV†
Total Carbohydrate	<1 g	<1%†
enhanced bioavailability water-dispersible turmeric (<i>Curcuma longa</i>) rhizome complex) providing 30% curcumin (180 mg)	600 mg	**

†Percent Daily Values (DV) are based on a 2,000 calorie diet.
**Daily Value not established.

Other ingredients: cellulose, maltose, hydroxypropyl methylcellulose (vegetable capsule), gum ghatti, ascorbyl palmitate, dextrin, citric acid, silicon dioxide

Contains no: salt, yeast, wheat, gluten, soy, dairy products, artificial colors, flavors, preservatives or ingredients of animal origin.

If pregnant, nursing, or taking prescription drugs, consult your healthcare professional prior to use.

Recommendations: Take as recommended by your healthcare professional. Typical dose is 2 capsules or more once per day for 7 days; may reduce dosage to 1 capsule per day thereafter, or as recommended by your healthcare professional.

Figure 2: Commercial curcumin supplement, the manufacturer's description of the product from the label.

Experimental and Results

The powdered contents were removed from a commercial curcumin capsule. The weight of the sample, with and without the capsules, was recorded. Approximately 150 mg of sample and control curcumin from *Curcuma longa* (Sigma Aldrich) were weighed into 15 mL centrifuge tubes. Ten grams of methanol were added, and the samples were sonicated for 30 minutes to extract the curcuminoids.



Following curcuminoid extraction, the centrifuge tube for the experimental sample (i.e. the sample from the nutraceutical bottle) contained a significantly larger insoluble pellet compared to the control sample, indicative of a number of water insoluble components in the experimental sample besides the soluble curcuminoids (Figure 3). The supernatants were removed and saved for analysis

Figure 3: Pellets obtained following extraction of curcuminoids with methanol and centrifugation for the experimental sample (left) and the curcumin control (right).

LC-MS was performed with an Agilent 1100 HPLC system. Prior to sample testing, three blank injections were performed to ensure that a stable baseline was established. The following ions were extracted from the positive mass spectra obtained for the experimental sample and the curcumin control: 369, 339, and 309 m/z. These m/z values correspond to curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively. An overlay of the LC-MS chromatograms of the three curcuminoid standards is shown in Figure 4.

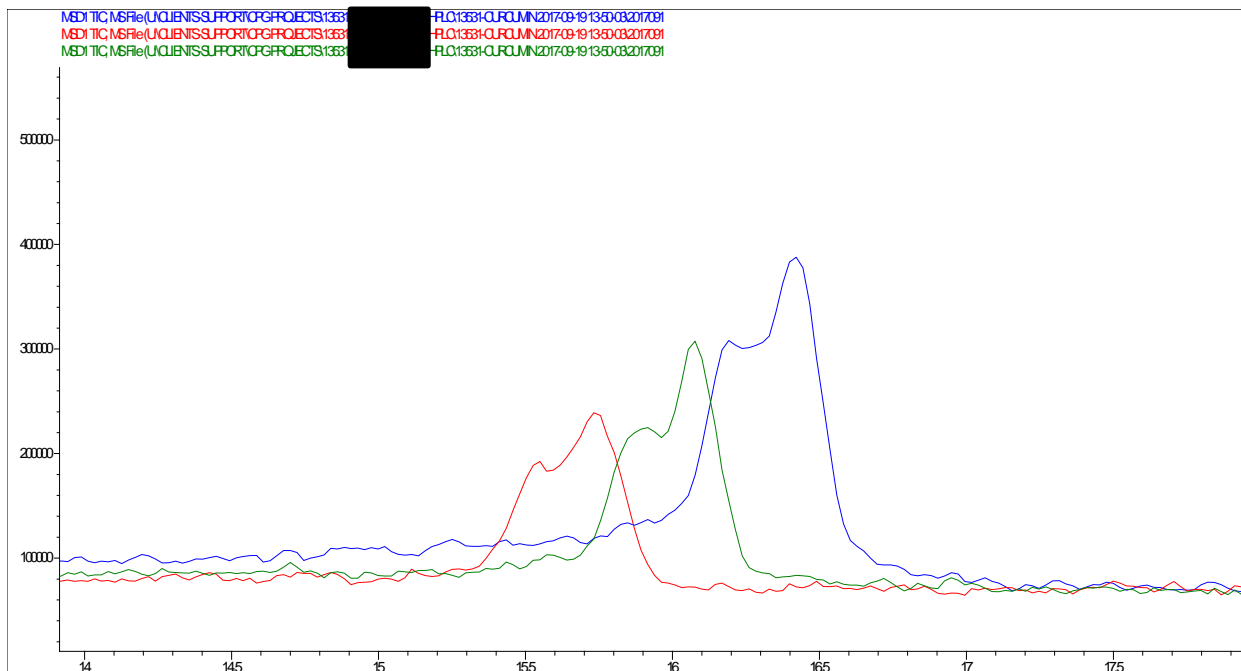


Figure 4: Overlay of LC-MS chromatograms for the three curcuminoid standards: curcumin (blue), demethoxycurcumin (green), and bisdemethoxycurcumin (red).

The concentration of each curcuminoid in the sample and control was determined by comparison to the calibration curves. An overlay of the MSD chromatogram and the extracted ion chromatograms for the experimental sample is shown in Figure 5. Only a minor peak was observed for bisdemethoxycurcumin relative to curcumin and, to a lesser extent, demethoxycurcumin. An overlay of the MSD chromatogram and the extracted ion chromatograms for the control is shown in Figure 6. As anticipated for *Curcuma longa*, peaks were readily observed for all three curcuminoids.

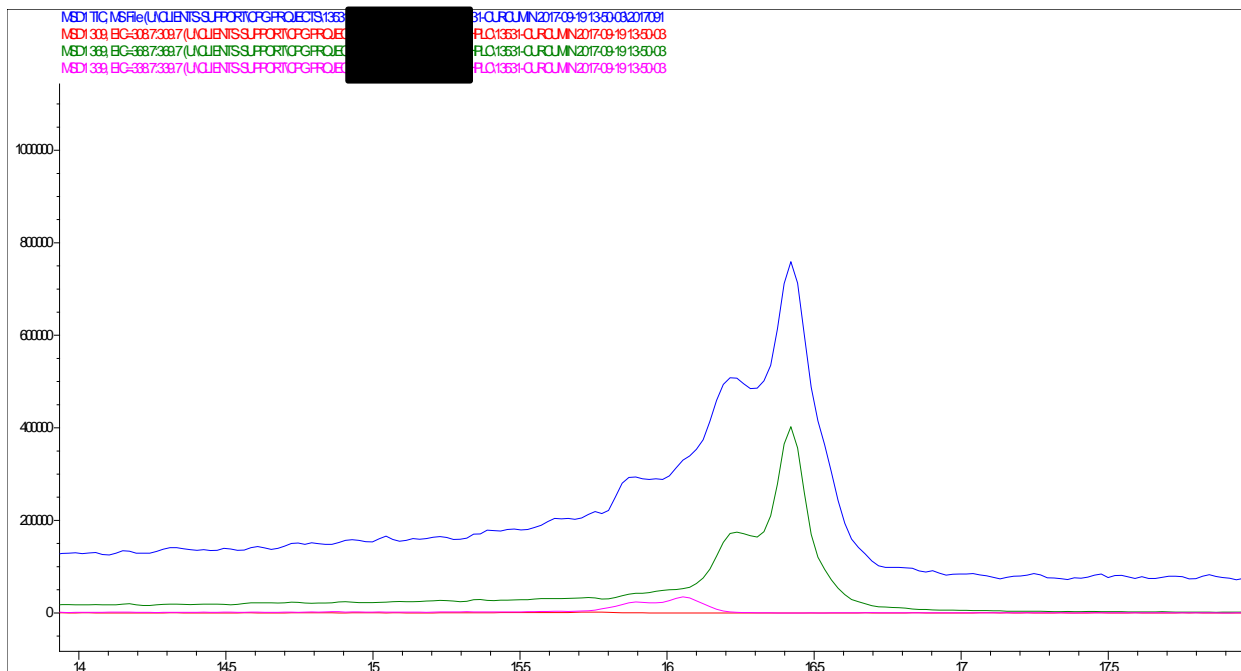


Figure 5: Overlay of the LC-MS chromatogram (blue) and select ion chromatograms for $m/z = 369$ (green), $m/z = 339$ (pink), and $m/z = 309$ (red) for the experimental sample.

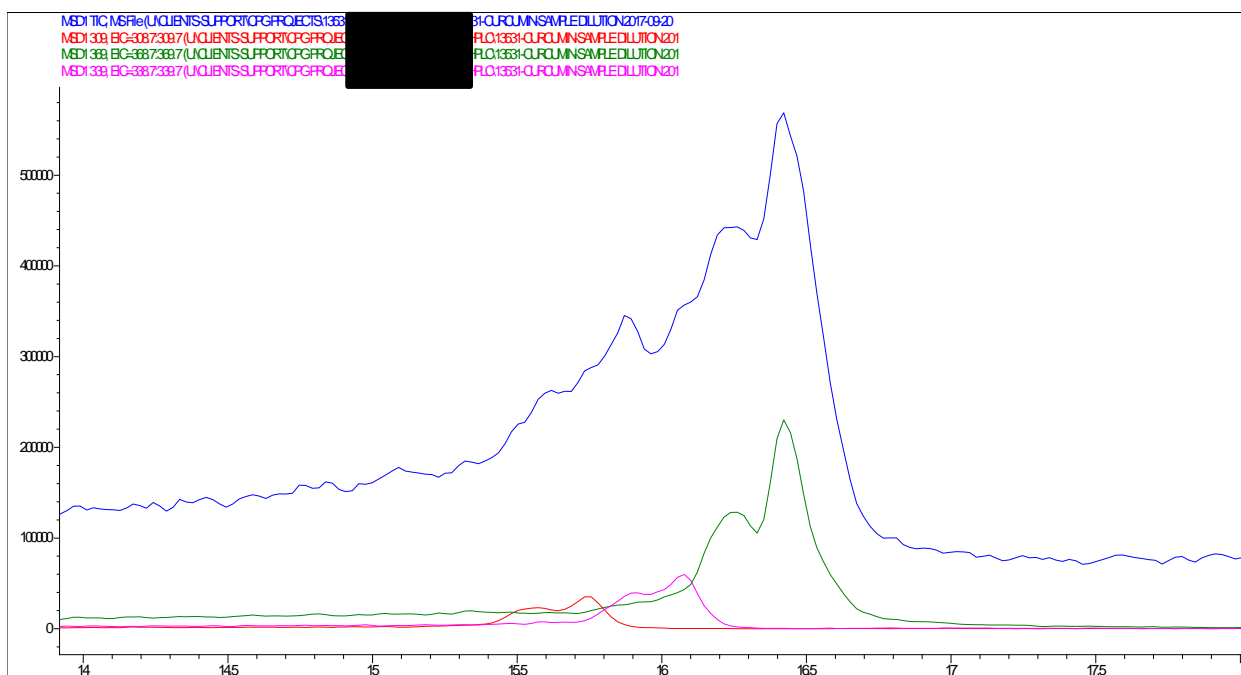


Figure 6: Overlay of the LC-MS chromatogram (blue) and select ion chromatograms for $m/z = 369$ (green), $m/z = 339$ (pink), and $m/z = 309$ (red) for the curcumin control.

The percentage of each sample comprised by curcuminoids, as well as the relative percentages of the three curcuminoids, is provided in **Table 1**. The same information is presented with the absolute percentages of the three curcuminoids in **Table 2**. The total percentage of curcuminoids in the control was high; and, based upon a literature review, the relative amount of the three curcuminoids was consistent with the amounts reported for curcuminoids typically extracted from *Curcuma longa*. Further, the percentage of

curcumin in the control was consistent with the percentage reported by Sigma Aldrich (66% actual \geq 65% claimed). In comparison, the total percentage of curcumin in the experimental sample was low compared to the percentage reported by the manufacturer (23% actual vs 30% claimed), while the amount of curcumin relative to demethoxycurcumin and bisdemethoxycurcumin was high. The relative amounts of curcuminoids in the experimental sample suggests additional purification of the curcuminoids was performed after extraction from *Curcuma longa* in order to isolate curcumin, the species known to possess the greatest anti-inflammatory and antioxidant activity based upon in vitro cellular studies.

Table 1: Relative curcuminoid content in the experimental sample and curcumin control.

	Experimental Sample	Curcumin Control
Percent curcuminoids	24.30	85.44
Relative percentage of curcumin	94.25	77.02
Relative percentage of demethoxycurcumin	5.67	14.10
Relative percentage of bisdemethoxycurcumin	0.08	8.87

Table 2: Absolute curcuminoid content in the experimental sample and curcumin control.

	Experimental Sample	Curcumin Control
Absolute percentage of curcumin	22.9	65.81
Absolute percentage of demethoxycurcumin	1.38	12.05
Absolute percentage of bisdemethoxycurcumin	0.02	7.58

The average weight of several experimental samples, with and without the surrounding capsule, are reported in Table 3, along with the total amount of curcumin found through LC-MS. Based upon the label of the experimental sample, each 600 mg capsule should contain 180 mg of curcumin (30%). As shown, the commercial curcumin supplement contained 40% less curcumin than advertised.

Table 3: Comparison between manufacturer values and values determined by CPG based on LC-MS.

	Capsule mass (mg)	Curcumin mass (mg)	% Curcumin
Determined by CPG	567.28 \pm 2.92	108.14 \pm 2.52	22.90
Reported by manufacturer	600	180	30

Conclusions

CPG scientists found that the percentage of curcumin contained within the nutraceutical supplement capsules was substantially less than what was claimed on the label. Due to the lack of regulatory control, there is no guarantee that a supplement will do or contains what is asserted on the bottle. However, manufacturers can voluntarily submit products for certification by either the United States Pharmacopeia (USP) or National Science Foundation (NSF). A seal from either of these groups indicates that the consumer can trust that the product has been verified to contain the ingredients listed.

This application note demonstrates CPG's capability to reformulate complex materials and to characterize and conduct purity analysis of raw materials. Industries served include pharma and nutraceuticals.