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Rapid and Sensitive Analytical Assessment of Curcuminoids and Three Common Turmeric Adulterants in a Single Run Using Liquid Chromatography and Tandem Mass Spectrometry

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ABSTRACT: In this study, we have developed and validated a liquid chromatography−tandem mass spectrometry (LC−MS/MS) method using a tandem sector quadrupole mass spectrometer to simultaneously detect and quantify three curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) and three of the most common food adulterants, particularly in turmeric, namely, metanil yellow, Sudan I, and Sudan red G using a single 11 min method. The method was validated for linearity, precision, and accuracy. The limit of quantification (LOQ) and the limit of detection (LOD) were determined as 1−2 and 0.1−0.2 ng/mL, respectively, for all six analytes. The method is accurate and precise, with intraday and interday accuracies ranging from 86.4 to 108.1 and from 86.0 to 107.7%, respectively, and intraday and interday coefficients of variation (CVs) ranging from 0.9 to 5.2 and 1.6 to 5.6%, respectively. A simple extraction procedure, easy sample preparation, high sensitivity, and rapid analysis make this method suitable for routine analyses. The validated method was applied to quantify curcuminoids in six commercial turmeric supplements. The amounts of curcuminoids varied significantly (∼30-fold) among the six supplements investigated in the present study. To demonstrate the practical applicability of the validated method, a commercial dietary turmeric supplement was spiked with 0.002−1% of adulterants, and their concentrations were accurately quantified. This method provides a rapid quantification of the three curcuminoids and three common adulterants simultaneously in a single run.

KEYWORDS: *turmeric, curcuminoids, adulteration, LC−MS/MS, dietary supplements, method development*

INTRODUCTION

Consumer acceptance and potential health benefits of natural products present in dietary supplements have gained significant attention globally over the past few decades.[1−4] For instance, turmeric, a plant-derived curry spice that originated from India and is currently consumed worldwide, has been well recognized for its health benefits determined by multiple *in vitro*, *in vivo*, animal, and human studies.[1,4] The health benefits of turmeric are primarily attributed to the three important curcuminoids present, namely curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC), which are present at 3−5% of the total turmeric powder. The average proportion of the three curcuminoids was determined to be CUR (77%), DMC (17%), and BMC (6%).[3,5] The United States Food and Drug Administration (FDA) recognizes curcuminoids as generally regarded as safe (GRAS).[6] Both *in vivo* and human studies suggest that CUR may act as anti-inflammatory, anticancer, anticardiovascular, antidiabetic, and antiaging agents.[5−7] In addition, several reports suggest that CUR may have a positive impact in treating wounds, arthritis, and Alzheimer's disease. Due to these advantages, many turmeric dietary supplements are available in the global markets with different formulations and various health claims, such as providing cardiovascular health benefits, enhancing cognitive functioning, promoting mobility and flexibility, and supporting joint health.[1−4,7] As per Statista (a company that specializes in the market and consumer data), the global turmeric market in 2012 was valued at ∼2.70 billion USD. In 2016, it was valued at ∼3.16 billion USD, and is projected to be ∼5.65 billion USD by 2027.[8]

Adulteration of food products and supplements has been a common and worrisome phenomenon in recent times, which occurs primarily for potential economic benefits.[9,10] Turmeric powder is reported to be adulterated in several ways, including with synthetic CUR and azo dyes such as metanil yellow, Sudan I, and Sudan red G (Figure 1). Azo dyes are synthetic aromatic compounds with an azo functional group (−N═N−), which is responsible for the coloring property of these dyes.[10,11] Natural products are widely used, mainly as coloring agents in several sectors such as food, cosmetics, textile, paper industries, and pharmaceutical companies.[10,12] There are approximately a total of 3000 azo dyes found, which constitute more than two-thirds of all dyes used in the textile and food industries.[10] The azo dyes, however, have been reported to cause severe health issues, including carcinogenicity, genotoxicity (chromosomal abnormalities), and genetic disorders in human beings.[10,13,14] Under anaerobic conditions, azo dyes reduce to form aromatic amines and cause carcinogenic risks to

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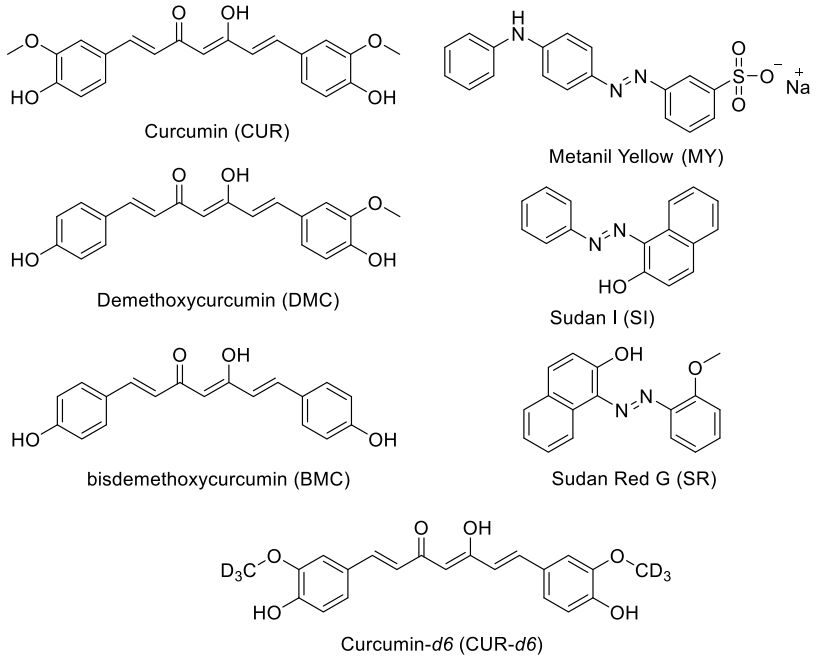

Figure 1. Structures of the three bioactive curcuminoids, curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC), and three common adulterants, metanil yellow (MY), Sudan I (SI), and Sudan red G (SR) investigated in the current study. Curcumin-*d6* (CUR-*d6*) was used as an internal standard.

human health.[9] Moreover, it has been reported that metabolic disorders were induced in rats after the administration of coloring dyes.[15] The Food and Agriculture Organization of the United Nations classified metanil yellow as a category CII carcinogen, and the International Agency for Research on Cancer classified Sudan I as a category III carcinogen.[9,16] Long-term consumption of metanil yellow causes tumor development, lymphocytic leukemia, and neurotoxicity.[9,17−19] Hence, it is very important to have fast and sensitive analytical methods that enable detection and identification of adulterants present in food products even at low concentrations.

Several studies have reported the detection and quantification of curcuminoids and adulterants either separately or combined, partially using analytical methods such as Fourier-transform infrared (FT-IR) spectroscopy,[20] Raman spectroscopy,[20] high-performance thin-layer chromatography (HPTLC),[21] high-performance liquid chromatography (HPLC),[22] and capillary electrophoresis (CE).[23] Although ultraviolet−visible spectroscopy (UV−vis), FT-IR, and Raman techniques provide rapid detection of analytes, the sensitivity, accuracy, and reproducibility (matrix interferences) remain challenging. HPLC, on the other hand, may provide reproducible results; however, sensitivity and matrix interference issues remain challenging. Because toxicity is concentration-dependent,[10,13] it is important to develop sensitive and rapid analytical methods capable of detecting multiple analytes and adulterants in a single run and quantify with better accuracy, reproducibility, and high throughput.

LC−MS/MS-based methods provide several advantages over other conventional analytical methods in terms of accuracy, sensitivity, and robustness, both qualitatively and quantitatively. Zhao reported the quantification of several color dyes in chilli powder and paste using LC−MS/MS.[24] Tsai reported an LC−MS/MS method to quantify 20 adulterants in chili powder.[16] However, these methods did not use a stable isotope-labeled (SIL) internal standard, which is not only useful to achieve accuracy and precision but also plays a significant role in compensating for the variable ionization effects caused by matrix components.[25,26] Moreover, none of these LC−MS/MS methods quantified curcuminoids and common food adulterants such as metanil yellow (MY), Sudan I (SI), and Sudan red G (SR) using a single assay. In addition, there is a need for better validated LC−MS/MS methods to quantify turmeric and related adulterants in terms of rapid, sensitive methods with a wider linearity range using a single assay.

In the current report, we developed and validated a fast LC−MS/MS method to simultaneously quantify three curcuminoids (CUR, DMC, and BMC) and three most commonly used adulterants (metanil yellow, Sudan I, and Sudan red G) in turmeric. We used deuterated curcumin-*d6* (CUR-*d6*) as an internal standard for the quantification of all analytes because stable isotopes of other analytes were either not available or very expensive. By using an LC−MS/MS method, we quantified the curcuminoid content in commercial turmeric dietary supplements. We further demonstrated the application of the method to the detection and quantification of three adulterants (metanil yellow, Sudan I, and Sudan red G) in a spiked turmeric supplement sample and determined the lowest detection limit that can be achieved.

MATERIALS AND METHODS

Chemicals. All chemicals and solvents were purchased from commercial sources and used without purification. CUR, DMC, BMC, metanil yellow (MY), Sudan I (SI), and Sudan red G (SR) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The internal standard CUR-*d6* was purchased from Toronto Research Chemicals (Toronto, ON, Canada). LC−MS grade water and acetonitrile were purchased from Fischer Scientific (Pittsburg, PA, USA). Deionized water (18 Ω) was obtained using a Millipore Milli-Q purification system (New Bedford, MA, USA). Extractions were carried out in 50 mL disposable polypropylene centrifuge tubes (Fisher Scientific, Pittsburgh, PA, USA). Poly(vinylidene difluoride)

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Table 1. LC−MS/MS Analytical Method for the Assessment of the Three Curcuminoids [Curcumin (CUR), Demethoxycurcumin (DMC), and Bisdemethoxycurcumin (BMC)] and Three Common Adulterants (Metanil Yellow, Sudan I, and Sudan Red)

						MS identification			
analytes	linearity range (ng/mL)	equation	r^2	LOD (ng/mL)	LOQ (ng/mL)	precursor ion m/z $[M + H]^+$	product ions (m/z)	SRM (m/z)	CE (V)
Curcumin (CUR)	1−5000	$y = 0.008804x + 0.0007969$	0.9931	0.1	1	369	177, 145	177	23
Demethoxycurcumin (DMC)	1−5000	$y = 0.007949x + 0.0004293$	0.9941	0.1	1	339	255, 147	255	16
*bis*Demethoxycurcumin (BMC)	2−5000	$y = 0.009245x + 0.0101000$	0.9947	0.1	2	309	147, 225	147	23
Metanil yellow (MY)	2−5000	$y = 0.005622x + 0.0010890$	0.9914	0.1	2	354	169, 157	169	27
Sudan I (SI)	2−2000	$y = 0.003075x + 0.0021620$	0.9941	0.2	2	249	93, 232	93	32
Sudan red G (SR)	1−5000	$y = 0.006008x + 0.0002940$	0.9935	0.2	1	279	123, 108	123	19

(PVDF) syringe filters with a pore size of 0.45 μm were purchased from National Scientific (Duluth, GA, USA). All turmeric dietary supplements (**TS1−TS6**) were purchased from commercial sources. The details of the turmeric supplements are provided in Table S1.

Preparation of Standard and Sample Solutions. The individual stock solutions of CUR, DMC, BMC, MY, SI, and SR were prepared at the following concentrations: CUR, DMC, BMC, and MY 1 mg/mL; SI and SR 0.5 mg/mL. All analytes were individually weighted accurately with an analytical balance and dissolved in methanol. All six standards were combined in equal proportions to make a final individual analyte concentration of 100 μg/mL, which was used as the primary stock solution. The CUR-*d*6 internal standard was prepared to a concentration of 1 mg/mL in methanol and was further diluted with methanol to 1 μg/mL. The concentration of IS was equal in all samples (100 ng/mL). Calibration curves were constructed in the range of 1−5000 ng/mL (1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL) for CUR, DMC, and SR; 2−5000 ng/mL for BMC and MY (2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL); and 2−2000 ng/mL for SI (2, 5, 10, 20, 50, 100, 200, 500, 1000, and 2000 ng/mL). The quality control (QC) samples were prepared in the same way as above at low, medium, and high concentrations of 5, 50, and 1000 ng/mL for CUR, DCM, BCM, and MY, and 5, 50, and 500 ng/mL for SI and SR.

Sample Extraction. Curcuminoids in turmeric dietary supplements were extracted using an optimized ultrasonic extraction method. Sample **TS4** was used as a model substrate for the extraction optimization. Four different organic solvents were evaluated to find the most suitable solvent for optimal extraction. Sample **TS4** (25 ± 0.1 mg) was placed in disposable 50 mL centrifuge tubes and dissolved in 25 mL of either methanol, ethanol, acetone, or acetonitrile in triplicate. Aliquots of each extract were analyzed at five different time periods (0, 5, 15, 30, and 60 min). All samples were vortexed for about 15 s prior to the extraction. All extracts were filtered through a 0.45 μm PVDF syringe filter prior to the analysis. Using the optimized extraction method, curcuminoids from six dietary supplements were extracted in triplicate. One-way ANOVA analysis was used to test for differences in total curcuminoids extracted in different solvents using JMP Pro 15.0.0 Statistical software (Cary, NC).

Instrumentation. *LC−MS/MS.* LC−MS/MS analysis was performed on a TSQ Vantage tandem sector quadrupole mass spectrometer (Thermo Scientific, Waltham, MA. USA) coupled with an Agilent 1290 UHPLC (Agilent Technologies, Santa Clara, CA, USA). The separation of the six analytes was carried out using an Agilent (Eclipse Plus) C18 column (1.8 μm, 4.6 × 50 mm) (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent guard column. The column was maintained at 35 °C, and the flow rate was 0.5 mL/min. Solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) were used as mobile phases. The sample injection volume was 10 μL. The total chromatography runtime was 11 min. All the analytes were eluted within 8.5 min. The solvent gradient was 0.0−8.0 min 50−85% B, 8.0−8.1 min 85−95% B, 8.1−9.6 min 95% B, 9.6−9.8 min 95−50% B, and 9.8−11 min 50% B. Mass spectra were acquired using heated electrospray ionization (HESI) in positive ion mode with a mass range of 50−1050 m/z. MS conditions were as follows: spray voltage, 4000 V, vaporizer temperature, 250 °C; capillary temperature, 250 °C; sheath gas pressure, 60 arbitrary units (au); auxiliary gas pressure, 10 au; sweep gas pressure, 0 au; and collision pressure, 1.0 mTorr. The data acquisition and analysis were performed using Xcalibur software 2.2 (Thermo Scientific, Waltham, MA, USA). The total 11 min MS acquisition was separated into five segments: (i) 0−1.5 min; (ii) 1.5−4.0 min for CUR, DMC, BMC, CUR-*d*6, and MY; (iii) 4.0−6.5 min; (iv) 6.5−9.0 min for SI and SR; and (v) 9.0−11 min. Only one full-scan event was carried out for segments (i,iii,v) with a scan time of 1.0 s, and three scan events (full scan, selected ion monitoring (SIM), and selected reaction monitoring (SRM)) were carried out for segments (ii,iv). For the full scan event, the mass range was m/z 50−1050 with a scan time of 1.0 s. For the SIM scan event, the scan time is 0.1 s and the scan width is 0.5; center masses: m/z 369 for CUR, m/z 339 for DMC, m/z 309 for BMC, m/z 354 for MY, m/z 249 for SI, and m/z 279 for SR; the quantification was done in the SRM mode. The scan width was 0.5 m/z and scan time was 0.1 s. Both Q1 and Q3 resolutions were set as 0.7 full width at half-maximum, and the CID gas pressure was 1 mTorr. All the SRM transitions are listed in Table 1.

Method Validation and Data Analysis. All calculations were based on the peak area response ratio of the analyte to its respective stable isotope-labeled internal standard (IS) CUR-*d*6. Data were collected and processed with Thermo Scientific Xcalibur 2.2 using the peak area ratio method. All statistics were performed using Microsoft Excel.

Linearity was measured by calibration curves that were constructed from 11−12 non-zero standards in order to determine the working analytical linearity range. Concentrations of the samples were calculated using linear regression analysis of a plot of calibration standard concentration versus the peak area ratio of standard to IS with a $1/x^2$ weighting factor. Sensitivity was measured by the limit of detection (LOD) and quantification (LOQ) for all analytes, which were calculated as per the International Council for Harmonization (ICH) guidelines.[27] The LOQ was defined as the lowest point on the calibration curve with a signal-to-noise ratio (S/N) ≥ 10, whereas LOD was measured by using a S/N ≥ 3. Accuracy and precision were determined using triplicates of three different concentrations (low, medium, and high) on two different days for intra- and interday analysis. Accuracy was measured as the % of calculated versus theoretical concentrations of the sample, whereas precision was measured as the relative standard deviation (RSD) of intra- and interday analysis.

HPLC Analysis with Diode Array Detection. The extraction efficiency of curcuminoids from the commercial turmeric dietary supplements was analyzed using an Agilent 1200 HPLC coupled with a Thermo Scientific MSQ single quadrupole mass spectrometer. An Agilent Eclipse Plus C18, 1.8 μm, 4.6 × 50 mm column equipped with an Agilent guard column, was used for the chromatographic separations. The column was maintained at 35 °C with a 0.7 mL/

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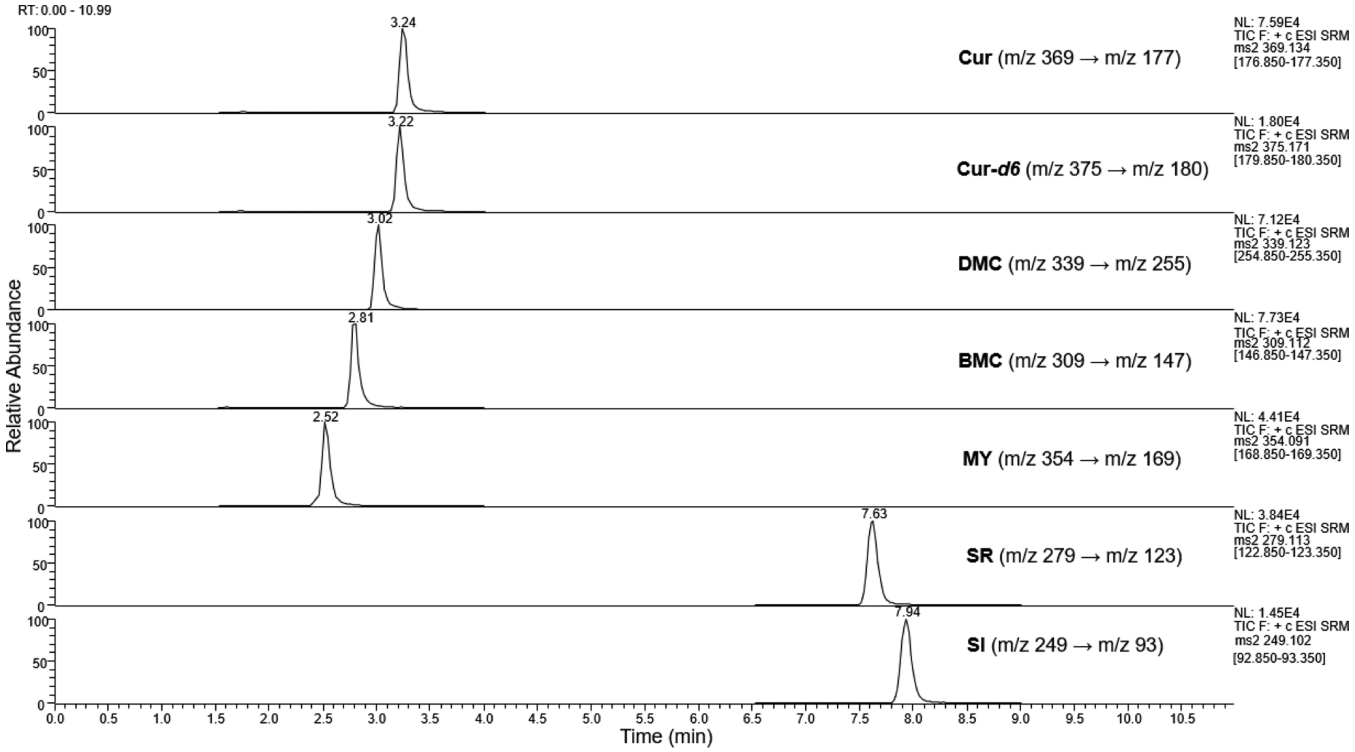

Figure 2. Representative selected reaction monitoring chromatogram for six analytes: three curcuminoids: curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC) and three common adulterants: metanil yellow (MY), Sudan I (SI), and Sudan red G (SR) and the internal standard deuterated curcumin-*d6* (CUR-*d6*).

Table 2. Accuracy and Precision Data for the Three Curcuminoids [Curcumin (CUR), Demethoxycurcumin (DMC), and Bisdemethoxycurcumin (BMC)] and Three Common Adulterants (Metanil Yellow, Sudan I, and Sudan Red)

		intra-day (n = 3)			interday (n = 6; 2 × 3)		
analyte	QC level (ng/mL)	mean ± SD (ng/mL)	accuracy %	CV %	mean ± SD (ng/mL)	accuracy %	CV %
curcumin (CUR)	5	5.30 ± 0.24	105.99	4.49	5.39 ± 0.21	107.73	3.92
	50	54.04 ± 1.44	108.08	2.67	53.69 ± 1.60	107.38	5.56
	1000	933.97 ± 16.32	93.40	1.75	928.80 ± 15.47	92.88	1.67
demethoxycurcumin (DMC)	5	5.37 ± 0.22	107.31	4.15	5.43 ± 0.29	108.62	5.40
	50	52.82 ± 1.73	105.64	3.28	53.56 ± 1.83	107.11	3.41
	1000	888.40 ± 23.06	88.84	2.60	891.66 ± 20.67	89.17	2.32
bis-demethoxycurcumin (BMC)	5	5.40 ± 0.26	107.96	4.87	5.30 ± 0.25	105.90	4.66
	50	52.19 ± 0.91	104.38	1.75	53.30 ± 1.96	106.61	3.67
	1000	873.00 ± 18.00	87.30	2.06	873.67 ± 21.12	87.37	2.42
metanil yellow (MY)	5	5.12 ± 0.27	102.35	5.21	5.23 ± 0.25	104.56	4.69
	50	53.22 ± 1.81	106.43	3.41	52.42 ± 1.84	104.84	3.51
	1000	877.37 ± 15.93	87.74	1.82	859.46 ± 30.26	85.95	3.52
Sudan I (SI)	5	5.27 ± 0.14	105.39	2.66	5.15 ± 0.26	103.09	4.99
	50	51.05 ± 1.62	102.09	3.17	51.08 ± 2.18	102.16	4.27
	500	443.61 ± 3.78	88.72	1.69	442.14 ± 7.16	88.43	1.62
Sudan red G (SR)	5	5.17 ± 0.07	103.33	1.28	5.22 ± 0.17	104.48	3.33
	50	50.97 ± 0.45	101.93	0.89	51.10 ± 2.13	102.20	4.17
	500	432.06 ± 5.50	86.41	1.27	429.94 ± 8.49	85.99	1.98

min flow rate. Solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) were used as mobile phases. The sample injection volume was 10 μL. The total chromatography run time was 15 min. The solvent gradient was 0.0−3.0 min 30−40% B, 3.0−10 min 40−60% B, 10.0−10.3 min 60−95% B, 10.3−13.3 min 95% B, 13.3−13.5 min 95−30% B, and 13.5−15.0 min 30% B. UV detector wavelengths of 425 and 280 nm were used for the detection, and 425 nm was used for the quantification of curcuminoids.

Quantification of Curcuminoids and Adulterants. The six samples of dietary supplements purchased from commercial sources claim different turmeric levels. These samples were extracted as per the experimental procedure. These samples were quantified in triplicate for the curcuminoids and adulterants.

Methanol was added to sample **TS4** (1 mg/mL), and the mixture of three adulterants was added to the solution at different concentrations (0.002−1% wt/wt). The resulting solutions were vortexed and extracted as described above, and the extracts were quantified using the TSQ Vantage.

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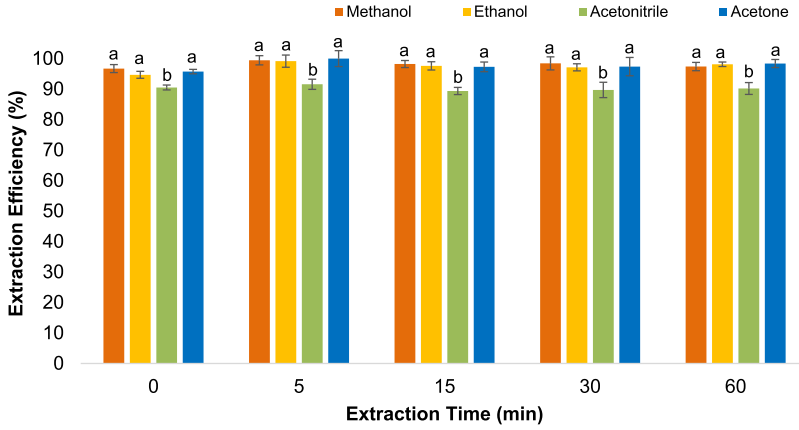

Figure 3. Analysis of extraction efficiency of curcuminoids in % from sample **TS4** at 1 mg/mL concentration at five extraction times (0, 5, 15, 30, and 60 min). All samples were extracted and analyzed in triplicates. The error bars show the ±standard deviation between three replicates. Levels not represented by the same letter (a or b) are significantly different based on one-way ANOVA analysis.

RESULTS AND DISCUSSION

LC−MS/MS Method Development and Validation. In the present study, MS ionization and SRM parameters for all analytes and internal standard were optimized using individual solutions. All analytes were optimized in the positive ion mode. In order to improve the sensitivity and maximize the performance of multianalyte MS/MS detection, we segmented the total analytical runtime into five segments based on the retention times of the analytes. All of the segments utilized a full scan. For segments 2 and 4, in which all of the analytes were detected, a full scan, SIM, and SRM were employed. The chromatography conditions were optimized using water (0.1% FA) and acetonitrile (0.1% FA) as mobile phases. Representative SRM transitions for analytes **CUR**, **DMC**, **BMC**, **MY**, **SI**, and **SR** and IS **CUR-*d*6** for separation of the six analytes are shown in Figure 2: **CUR** (m/z 369 → m/z 177, RT 3.2 min), **DMC** (m/z 339 → m/z 255, RT 3.0 min), **BMC** (m/z 309 → m/z 147, RT 2.8 min), **MY** (m/z 354 → m/z 169, RT 2.5 min), **SR** (m/z 279 → m/z 123, RT 7.6 min), and **SI** (m/z 249 → m/z 93, RT 7.9 min) along with **CUR-*d*6** IS (m/z 375 → m/z 180, RT 3.2 min). Table 1 shows the retention times and SRM parameters for all analytes studied.

The developed LC−MS/MS method was validated for linearity, accuracy, precision, and sensitivity according to the ICH guidelines.[27] Calibration curves for all the analytes individually were plotted using linear regression analysis of standard concentration versus the peak area ratio of standard to IS with a $1/x^2$ weighting factor. All the analytes showed excellent linearity with r^2 of ≥0.99. Typical regression equations are shown in Table 1. The linear ranges for all analytes were 1000−5000-fold ranges of concentration. Accuracy (% bias) and precision (RSD) were measured by using six replicates (triplicates on two different days; intra- and interday) of the calculated concentrations of each analyte quality control (QC) sample at three different concentration levels (5, 50, and 1000 ng/mL for **CUR**, **DMC**, **BMC**, and **MY**; 5, 50, and 500 ng/mL for **SI** and **SR**). The accuracy and precision data are presented in Table 2; intraday accuracy and precision were in the ranges of 86.4−108.1 and 0.9−4.9%, respectively. Similarly, the accuracy and precision for interday were in the ranges of 86.0−108.6 and 1.6−5.6%, respectively.

Recently, Wu and co-workers developed a sensitive analysis of curcuminoids via micellar electrokinetic chromatography with laser-induced native fluorescence detection and mixed micelle-induced fluorescence synergism, which could enhance the signals of three curcuminoids by 77-, 57-, and 47-fold for CUR, DMC, and BMC, respectively.[28] The authors determined the linearity range for CUR and DMC as 0.1−50, and 0.01−5 μg/mL for BMC. The LODs, calculated from a signal-to-noise ratio of 3, were 4.1, 2.6, and 0.4 ng/mL for CUR, DMC, and BMC, respectively. Similarly, in a separate study, Girme and co-workers reported the assessment of adulteration of turmeric extracts with synthetic CUR using HPLC and HPTLC targeting the synthetic intermediate CIMP-1, that is, (1*E*,4*Z*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) hexa-1,4-dien-3-one.[29] The authors calculated that the linearity range for CIMP-1 was 1−20, 10−800 μg/mL for CUR, 1.75−140 μg/mL for DMC, and 0.5−40 μg/mL for BMC, respectively. The LOD and LOQ for the four analytes (CIMP-1, CUR, DMC, and BMC) were determined as 0.0623, 0.265, 0.12, and 0.076 μg/mL, and 0.1899, 0.804, 0.35, and 0.23 μg/mL, respectively. The LOD and LOQ data from the present study for all the analytes are presented in Table 1. The LODs were in the range of 0.1−0.2 ng/mL, and LOQs were in the range of 1−2 ng/mL for all the analytes, respectively. Similar variations between LOD and LOQ values for curcuminoids have been reported in the literature.[30,31] The low LODs and LOQs highlight the sensitivity of the current method, as sensitivity is very important to quantify the toxic adulterants at low concentrations.

Extraction of Curcuminoids from the Six Commercial Turmeric Supplements. There have been several extraction procedures reported in the literature for the extraction of curcuminoids from turmeric and related products.[4] Most of the reported procedures lack extraction optimization details. In the current study, an ultrasonication-assisted extraction procedure was optimized by testing five different time periods (0, 5, 15, 30, and 60 min) with four different organic solvents, namely, methanol, acetone, acetonitrile, and ethanol. Sample **TS4** was used as the model substrate for the optimization of the extraction process. For this purpose, an HPLC with diode array detection method was developed, as shown in the experimental section. The area under the curve was determined for three curcuminoids and summed to get the total area. The data showed that methanol, acetone, and ethanol were able to extract ∼90% of the curcuminoids without any ultrasonication. Moreover, 100% of curcuminoids were extracted with just 5 min of ultrasonication (Figure 3). Among the solvents studied,

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Table 3. Quantification of the Sum of the Three Identified Curcuminoids in Six Different Turmeric Dietary Supplements[a]

	calculated amount (mg/capsule)				
sample #	CUR	DMC	BMC	total curcuminoids	amount (mg) of curcuminoid/capsule as specified by the vendor
TD1	32.30 ± 0.43	8.70 ± 0.10	2.98 ± 0.12	43.98 ± 0.64	47.50
TD2	5.51 ± 0.09	1.28 ± 0.02	0.18 ± 0.003	6.96 ± 0.11[b]	NA
TD3	37.70 ± 0.40	3.43 ± 0.06	1.41 ± 0.02	42.54 ± 0.43	47.50
TD4	32.07 ± 0.50	8.66 ± 0.16	6.31 ± 0.21	47.04 ± 0.62	47.50
TD5	1.17 ± 0.04	0.37 ± 0.02	0.29 ± 0.02	1.83 ± 0.07[b]	NA
TD6	2.65 ± 0.04	0.87 ± 0.001	0.48 ± 0.01	4.27 ± 0.05[b]	NA

[a]All analyses were done in triplicates. [b]mg/500 mg powder, NA: not available.

acetone (100%), methanol (99.5%), and ethanol (99.2%) provided the maximum extraction efficiency. Acetonitrile was the least efficient (~91.58%) solvent in terms of extracting curcuminoids from a turmeric sample. Based on these results, we selected methanol as the choice of solvent for the extraction process as it has been widely used in curcumin-related research and analysis.[4] Moreover, in the current study, all the calibration standards and quality control samples were prepared in methanol. However, one can also use ethanol or acetone as a solvent for the extraction of curcuminoids.

Six commercial dietary supplements were used to determine the concentrations of curcuminoids and to possibly detect the presence of adulterants. Among the six supplements, four of them were capsules, and two of them were powders. All of the supplements claimed to contain turmeric root or turmeric extract; however, only three of the supplements (all were capsules) claimed to have turmeric extract with standardized 95% curcuminoids; each had about 47.5 mg/capsule. The optimized extraction method was used to extract all curcuminoids and potential adulterants. No adulterants were present in any of the supplements. Table 3 shows the curcuminoids' quantification data. Supplements **TS1**, **TS3**, and **TS4** had significantly higher curcuminoids compared to supplements **TS2**, **TS5**, and **TS6**. **TS1** had 43.3 mg/capsule, and **TS3** and **TS4** contained 42.5 mg/capsule and 47.0 mg/capsule, respectively.

As none of the six supplements tested in the study were adulterated, to demonstrate the practical application of the current LC−MS/MS method for quantifying the adulterants accurately and precisely, we spiked sample TS4 with a three-adulterant mixture (MY, SI, and SR) and extracted as described earlier using the optimized extraction method. Five different concentrations of adulterants 0.002, 0.02, 0.1, 0.5, and 1% (wt/wt %) were used for the method evaluation. Table 4 shows the quantification data for three adulterants. The data showed that the current method quantifies with excellent accuracy (97.3−100.3%) and precision (RSD 0.9−7.8%) for the lowest amounts of 0.002% (wt/wt %) of adulterants we studied.

Two publications by Dixit and co-workers reported that no adulteration was observed in the limited number of branded samples tested in their study.[32,33] However, over 15% of the loose powder commonly used as food spice exhibited the adulteration problem. It is important to be able to identify and quantify the adulterants accurately and precisely at very low concentrations as they are known to have genotoxic, carcinogenic, and other toxic effects on human health.

In conclusion, we have developed and validated an LC−MS/MS method to quantify three bioactive curcuminoids (CUR, DMC, and BMC) and three of the most common food adulterants (MY, SI, and SR) using a single 11 min method.

Table 4. Detection of Three Adulterants in a Single Model Dietary Supplement

adulteration %	amount of adulterant added (theo. conc.) (ng/mL)	amount calculated (ng/mL)	mean % accuracy	SD	% CV
		Metanil Yellow			
0.002%	4	3.89	97.29	0.03	0.9
0.02%	40	41.88	104.70	0.24	0.6
0.10%	200	176.06	88.03	0.44	0.2
0.50%	1000	997.60	99.76	8.87	1.1
1.00%	2000	1820.57	91.03	8.43	0.5
		Sudan I			
0.002%	4	4.01	100.31	0.31	7.8
0.02%	40	35.04	87.61	1.45	4.1
0.10%	200	176.51	88.26	6.47	3.7
0.50%	1000	1058.86	105.89	10.12	1.0
1.00%	2000	2154.1	107.7	55.26	2.6
		Sudan Red			
0.002%	4	3.96	98.95	0.14	3.6
0.02%	40	34.75	86.87	1.64	4.7
0.10%	200	174.27	87.14	3.73	2.14
0.50%	1000	928.53	92.85	23.76	2.6
1.00%	2000	1874.89	93.74	75.53	4.0

The validated LC−MS/MS method is a suitable method for rapid analysis with excellent accuracy and precision. The method was proved to be sensitive and able to quantify all the analytes we studied with LODs of 0.1−0.2 ng/mL and LOQs of 1−2 ng/mL. Moreover, the current method has linear ranges of 1−5000 ng/mL for CUR, DMC, and SR, 2−5000 ng/mL for BMC and MY, and 2−2000 ng/mL for SI. To the best of our knowledge, this is the first LC−MS/MS-validated method for three curcuminoids and three common adulterants in a single assay with a broad linearity range. The validated method was further used to successfully quantify curcuminoids in commercial dietary supplements extracted using an optimized method. More importantly, using the current LC−MS/MS method, three of the most common food adulterants MY, SI, and SR with which turmeric samples were spiked were successfully quantified even at very low amounts of 0.002% (wt/wt). This rapid method can be used to screen and quantify trace quantities of three bioactive curcuminoids and three adulterants in a single run.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.1c00356.

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Overlay of representative extracted ion chromatograms and details of the six commercial turmeric dietary supplements (PDF)

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Notes

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