Curcuminoids in Turmeric Roots and Supplements: Method Optimization and Validation

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Abstract *Curcuma longa* L. rhizomes are used extensively as a spice in food preparations and dietary supplements for their anti-inflammatory and antioxidant properties. An expert review panel (ERP) evaluated analytical methods for the quantitation of individual curcuminoids for the purpose of identifying a method for official method status. It was requested that several modifications be undertaken to improve method performance prior to subjecting the chosen method to a single-laboratory validation. Two separate Plackett-Burman factorial studies were used to identify factors that contributed to the chromatographic separation and extraction of curcuminoids. Significant factors were further optimized to produce the improved HPLC method for curcuminoid separation. This method was then subjected to a single-laboratory validation according to the AOAC International guidelines for linearity, detection limits, precision, and accuracy. The two most significant factors impacting the quantitation of curcuminoids were column temperature and extraction solvent, which were optimized to 55 °C and 100 % methanol, respectively. The validation was performed on 12 raw materials and finished products containing turmeric roots. The method precision was reported using HorRat values which were within recommended ranges of the AOAC guidelines. Overall accuracy of the method was assessed at three separate levels for each analyte and ranged from 99.3–100.9 %. The validated method is suitable for quantitation of individual curcuminoids in turmeric raw

materials and finished products and is recommended for consideration as an official method by the AOAC International.

Keywords *Curcuma longa* L. · Turmeric · HPLC · Single-laboratory validation · Curcuminoids · Dietary supplements

Introduction

Curcuma longa L., or turmeric, is a perennial herb that originates from India. It is part of the family Zingiberaceae, contains thick rhizomes that are a bright yellow, and is commonly used as spices in many food preparations such as curries. Turmeric is also available as a dietary supplement for its anti-inflammatory and antioxidant activities (Motterlini et al. 2000; Ramesewak et al. 2000; Jurenka 2009). Several studies have also confirmed anticancer activities of turmeric constituents (Bar-Sela et al. 2010; Yu and Huang 2010). With the increasing global demand for turmeric products in foods and dietary supplements, there has been an increase in adulteration of turmeric products. The most common adulterant is metanil yellow, a yellow dye, with similar color and consistency to turmeric, while its consumption is thought to be potentially carcinogenic (Dixit et al. 2008, 2009).

The components responsible for the yellow color in turmeric are the diarylheptanoid derivatives or curcuminoids. The three major curcuminoids found in turmeric are curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) (Hiserodt et al. 1996). These curcuminoids are typically measured in turmeric-based extracts and dietary supplements for product strength. While the yellow pigment of curcuminoids may suggest that simplified spectrophotometric measurements for total curcuminoids could be used, it is essential to ensure that the three curcuminoids are

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identified, confirmed, and quantified after solvent extraction to ensure sufficient quality control of products (Taylor and McDowell 1992). The most suitable method to quantitation of curcuminoids is with HPLC with UV-Vis and/or mass spectrometric detection (Jayaprakasha et al. 2002; Jiang et al. 2006; Wichitnithad et al. 2009).

Adequate chemical characterization and verification of botanical products used in clinical studies has been found to be insufficient by not including product strength information, relying solely on manufacture specifications or using nonvalid method to quantify the active ingredients (Khan 2006; Betz et al. 2007). This lack of information makes it impossible to reproduce clinical study results. In addition to this, there is insufficient availability of official, validated methods for clinicians, manufacturers, and regulatory agencies to provide accurate determination of the declared ingredients and/or active components in the products (Betz et al. 2007). Therefore, the development, validation, publication, and acceptance as official methods for analytical determinations for active ingredients in dietary supplements are essential to improve product quality and clinical research outcomes.

Based on the lack of available reliable analytical methods, sales volumes, uses, and safety of turmeric products, the NIH ODS Dietary Supplements Presidential Task Force identified turmeric as a high priority supplement requiring a validated method for the determination of curcuminoids in raw materials and finished products. An AOAC expert review panel (ERP) met in 2010 and evaluated 38 turmeric methods for curcuminoid quantification. One method was selected for further consideration for single-laboratory validation, although due to poor performance on some matrices and peak shape in chromatograms, several modifications were recommended prior to the validation of the method (Solyom 2012).

The objectives of this work were to address the modifications suggested by the ERP for turmeric method optimization by using factorial studies to guide the optimization. Factors related to the chromatography and extraction were evaluated, then further optimization of the major factors was completed. The optimized method was further subjected to single-laboratory validation according to the AOAC International guidelines to establish the method's performance characteristics.

Materials and Methods

Reagents

HPLC grade methanol and acetonitrile were purchased from VWR International (Mississauga, ON, Canada). HPLC grade formic acid was purchased from Fisher Scientific (Ottawa, ON, Canada). Water was deionized using a Barnstead water purification system from Fischer Scientific and further filtered through 0.22 µm nylon filters.

Reference Materials

The primary grade reference standards for curcumin (97.7 % purity), demethoxycurcumin (92.1 % purity) and bisdemethoxycurcumin (99.4 % purity) were supplied by ChromaDex Inc. (Irvine, CA). The standards were stored at 4 °C in a desiccator prior to use. Metanil yellow (70 % dye) was purchased from Sigma-Aldrich (Oakville, ON, Canada).

qNMR Purity Assessment

To assign purity, quantitative NMR (qNMR) analysis was performed on the purchased curcuminoid reference standards using a Varian Mercury Vx spectrometer operating at 400.13 MHz for ¹H. Samples were dissolved in acetone-D₆ and analyzed by standard proton NMR spectrometry for identification and qNMR for quantitative analysis (Pauli et al. 2005).

Study Materials

Whole dried turmeric roots (*Curcuma longa* L.) used in both the optimization and validation studies were collected in India by Sebastian Pole in 2006 and were obtained from the American Herbal Pharmacopeia (Scotts Valley, CA). These rhizomes were verified by two separate labs using HPTLC and were evaluated for microscopic and macroscopic identification. A second source of turmeric rhizomes used only in the validation study was collected in Kauai in 2013 by Diane Ragone of the National Tropical Botanical Garden (Kalaheo, HI), accession number 970275. These rhizomes were freeze dried. Both rhizomes were ground with a Retsch centrifugal mill fitted with a 0.25-mm sieve. Ground materials were stored in polypropylene containers protected from light at room temperature. Several single and multicomponent dietary supplements were purchased from suppliers including four capsules, one tablet, two tinctures and two softgels, and a bulk turmeric extract. A summary of these products can be found in Table 1.

Method Optimization

Initial investigations into the major factors influencing the analysis of curcuminoids in turmeric extracts were determined using Plackett-Burman factorial designs. A three-factor design was used for chromatographic separation optimization, and a seven-factor design was used for the extraction optimization as described below.

Chromatographic optimization initially focused on column selection. Columns were tested for their ability to separate a mixed standard solution containing 100 µg/mL of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The following four different columns with different packing and

Table 1 Test samples used in optimization and validation studies

Identification no.	Test sample description
294	Alcohol-based tincture, turmeric rhizome
299	Alcohol-based tincture, turmeric
297	Multicomponent softgel
298	Turmeric extract softgel
8019	Dried turmeric roots
8020	Freeze-dried turmeric roots
296	Boswellia and turmeric extract capsule
300	95 % curcumin turmeric rhizome capsule
301	95 % curcumin, <i>Piper nigrum</i> fruit extract capsule
293	Turmeric, black pepper fruit extract tablet
295	Turmeric root extract tablet
4139	<i>C. longa</i> root extract 85 % curcumin

dimensions were tested: Phenomenex Luna[®] C18(2) HST 2.6 μ m, 2.0 $\times$ 100 mm; Phenomenex Kinetex[®] C18 2.6 μ m, 4.6 $\times$ 100 mm, Agilent Zorbax SB-Aq 2.6 μ m, 4.6 $\times$ 100 mm; and Phenomenex Kinetex[®] C18 2.6 μ m, 2.1 $\times$ 30 mm. Each column was evaluated based on observed back pressure, solvent consumption, peak shape, and peak resolution.

A three-factor, two-level Plackett-Burmann factorial study was performed to evaluate the effect the column temperature and addition of acid in the mobile phases had on peak shape, peak resolution, column backpressure, and peak shape. The levels for column temperature were 35 and 60 °C, and the levels of formic acid addition in the mobile phases were 0 and 2.0 % and performed according to the study in Table 2. A third factor, performance of analysis in lit or dark room, was used as a dummy. Following the interpretation of the factorial results, further optimization was performed for column temperature and acid concentration in the mobile phases. Additionally, various gradient and flow rate conditions were evaluated to optimize run time and back pressure.

In the extraction optimization, a seven-factor, two-level Plackett-Burmann factorial study was performed to evaluate several parameters that could impact the extraction of curcuminoids. The parameters and the levels are listed in Table 3. The turmeric root sample and a commercial extract were both

Table 2 Factorial design for the three-factor chromatographic optimization

Runs	Column temperature	Formic acid in mobile phase	Lights on/off
1	–	–	+
2	+	–	–
3	–	+	–
4	+	+	+

Table 3 Seven factors investigated in the extraction optimization

Factor	Low	High
Methanol concentration (%)	70	100
Formic acid concentration (%)	0	1
Sonication time (min)	10	60
Sonication temperature (°C)	0	50
Sample size (mg)	75	300
Analyst (dummy)	1	2
Lights on/off (dummy)	On	Off

used. The specified amount of sample was extracted with 25 mL of solvent specified in the factorial design, either 70 or 100 % methanol. The solution was sonicated for the allotted time followed by centrifugation at 5000 rpm for 5 min. The extract was filtered through a 0.22- μ m polytetrafluorethylene (PTFE) filter into an HPLC vial prior to analysis. For each run, the concentrations for each of the three curcuminoids were determined using a calibration curve prepared from the mixed standard solutions. Following analysis of the factorial study, further optimization of the extraction was performed.

Sample Preparation

For sample preparation, the contents of dietary supplements were combined prior to sample extraction to ensure homogeneous mixtures. For capsules, the contents of 20 capsules were combined and mixed thoroughly. For tablets, 20 tablets were ground in a coffee grinder and mixed thoroughly. For softgels, 20 dosage units were combined and mixed thoroughly. All solid samples were stored in a polypropylene container protected from light prior to weighing of samples.

For raw materials, bulk powdered extracts, capsules, and tablets, 75 mg of material was extracted with 25 mL of methanol. The samples were extracted with a wrist-action shaker for 15 min followed by centrifugation at 5000 rpm for 5 min. Several samples with high curcuminoid contents were diluted 1:10. Samples were filtered with a 0.22- μ m PTFE filter into an HPLC vial prior to analysis. For softgels, 200 mg of material was extracted with 25 mL of methanol according to the procedure summarized above. Tinctures were mixed by inversion and diluted 1:10 with methanol and mixed. The samples were filtered through a 0.22- μ m PTFE filter into an HPLC vial prior to analysis.

Stock Solution Preparation

Individual 1000 μ g/mL stock solutions of each analyte (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) were prepared in separate 10.0 mL volumetric flasks using methanol as the diluent. Seven mixed calibration standard solutions were prepared in amber vials using the standard

stock solutions and diluted with methanol. Curcumin concentration ranged from 5 to 300 µg/mL, demethoxycurcumin ranged from 1 to 100 µg/mL, and bisdemethoxycurcumin ranged from 1 to 120 µg/mL. All stock solutions were stored at −20 °C protected from light when not in use.

HPLC Analysis

An Agilent 1200 Series Liquid Chromatograph equipped with a quaternary pump capable of operating at 400 bar and degasser, temperature controlled column compartment, autosampler, and UV-Vis diode array detector was used. The separation was achieved on a Kinetex® C18 2.1 × 30 mm, 2.6 µm column (Phenomenex, Torrance CA) with an ULTRA C18 SecurityGuard™ precolumn. The mobile phase was composed of (A) 0.1 % formic acid in water and (B) 0.1 % formic acid in acetonitrile with a flow rate of 1.4 mL/min (0–4.1 min). During the column flushing and equilibration (4.1–6.0 min), the flow rate is increased to 1.75 mL/min to increase sample throughput. The mobile phase gradient was as follows: 0–1 min, 28 %B; 1–2 min, 28–30 %B; 2–4 min, 30%B; 4–4.1 min, 30–50 %B; and 4.1–6.0 min 50 %B. The column temperature was 55 °C and with an injection volume of 0.8 µL. The UV-Vis detector was monitored at 425 nm for the detection of curcuminoids. Data was processed using the ChemStation software.

Method Validation

Precision was determined by preparing four replicates of each of the test samples on three separate days for a total of 12 replicates. The within-day, between-day, total precision, and HorRat value for each of the individual curcuminoids were then calculated for all the test materials investigated.

The calibration standards were injected three times throughout each run during the precision testing. From these mixed standards, individual seven-point calibration curves were generated for each analyte and observed for linearity. Linear regression analysis was used to determine the equation of the lines for each curve, and the correlation coefficient (r^2) for each of the curves was determined.

The detection limit for each of the analytes was determined using the protocols described by the EPA for determination of the method detection limit (Environmental Protection 2002). Two sets of seven replicates containing low concentrations of the mixed reference solutions were analyzed, and the method detection limit (MDL) was determined as the product of the standard deviation of the replicates and the t -statistic at $\alpha=0.01$ and $N-1$ degrees of freedom. The limit of quantitation was calculated as ten times the standard deviation of the replicates.

Recovery and accuracy were determined by preparing a homogeneous mixture of 99 % w/w maltodextrin and 1 %

w/w magnesium stearate to mimic a dried capsule extract. The curcuminoid stock solutions were spiked onto 75 mg of this material in triplicate at the following three levels: 0.3, 3.0, and 6.0 % w/w total curcuminoids, when extracted with 25 mL of methanol. These levels were chosen to bracket the calibration curves and the analyte ratios found in the test samples. The samples were then processed and analyzed as per the procedures described above.

Data Analysis

Individual curcuminoids were quantified for test materials in milligrams per grams or micrograms per milliliters using external calibration. Microsoft Excel was used to evaluate the factors in the Plackett-Burman factorial studies and for all validation data including Horwitz value, relative standard deviation, and calibration curve generation.

Results and Discussion

Chromatographic Optimization

The following four columns were investigated for the separation of curcuminoids: Luna® C18(2) HST 2.6 µm 2.0 × 100 mm (Phenomenex, Torrance, CA), Kinetex® C18 2.6 µm 4.6 × 100 mm, Kinetex® C18 2.6 µm 2.1 × 30 mm, and Zorbax SB-Aq 2.6 µm 4.6 × 100 mm (Agilent Technologies). The chromatography was optimized for resolution, low backpressure for use with traditional HPLC systems, and solvent usage. The separation on the long 100 mm columns had high solvent consumption, and in two columns, the peak shapes were not desirable. The separation on the 30-mm column met all the specified criteria and was selected for further optimization.

A three-factor Plackett-Burman factorial was used to optimize the chromatographic conditions, as summarized in Table 2. The curcuminoids from the raw materials were extracted, and the column temperature and mobile phase composition were varied according to the study. The chromatograms were evaluated for theoretical plates, peak tailing, and resolution in order to determine the major factors. It was observed that temperature has a large impact on all evaluation criteria. Further optimization evaluating the same criteria was used to optimize the separation with the final column temperature at 55 °C and 0.1 % formic acid added to both mobile phases. The flow rate, gradient, and injection volume were also optimized. Due to the poor solubility of curcuminoids in water, the injection volume was set to 0.8 µL. The final chromatogram of turmeric standards and ground rhizome are summarized in Figs. 1 and 2.

Fig. 1 Chromatographic separation of the curcuminoid standards bisdemethoxycurcumin, demethoxycurcumin, and curcumin at 425 nm

Extraction Optimization

A seven-factor Plackett-Burman study was used to determine the significant factors in the extraction of curcuminoids from turmeric raw materials as summarized in Table 3. For each injection, the final concentration of the three major curcuminoids was compared. A factor plot has been generated comparing the high to low data sets for all three curcuminoids in Fig. 3. A larger slope is an indicator of a factor that has an impact on the extraction. If there is a minimal slope, this factor does not impact the extraction and therefore is no longer investigated in the optimization. Based on the factors assessed in Fig. 3, there is a large slope from the concentration of methanol where 100 % methanol gave the higher concentration of curcuminoids and was chosen as the optimized extraction solvent. The other factors were not significant in the extraction optimization and therefore were not investigated further.

Metanil yellow was spiked into a solution containing curcuminoid standards. The color of the methanol solution was distinctly different from the turmeric extracts as the

metanil yellow appeared bright red/orange compared to the yellow from the curcuminoids. To further confirm that metanil yellow was present, a large peak was present in the HPLC chromatogram eluting prior to the curcuminoids as shown in Fig. 4. The addition of metanil yellow impacted the precision of the extraction, and solubility issues were observed; therefore, a guard column was added to the chromatography setup to increase the column life in case metanil yellow is present in turmeric products.

According to the recommendations of the ERP, this method is to be optimized for a variety of matrices, such as tablets, tinctures, softgels, capsules, bulk extracts, multicomponent products, and raw materials. These materials were sourced for extraction optimization, to ensure adequate chromatographic separation and to assess the method suitability. Extraction efficiency was evaluated for all matrices, where it was observed that softgels were the most complex matrix and gave lower recoveries; therefore, the method of extraction was further optimized. Wrist-action shaking and sonication were compared for a 15-min extraction of ground rhizomes and turmeric softgels. Although no significant difference was observed between the two extraction methods, the results were slightly higher with the wrist-action shaker, and the variance between samples was considerably lower, therefore improving the precision of the extraction. Due to the variance in curcuminoid content between different matrices, the sample size varies depending on the matrix as described in the sample preparation section of the method validation.

Fig. 2 Chromatographic separation of the curcuminoids in turmeric roots detected at 425 nm

Method Validation

The optimized HPLC method for individual curcuminoids was subjected to a single-laboratory validation according to the AOAC International guidelines (AOAC International 2013). The validation was used to determine the performance

Fig. 3 Factor plots for the three curcuminoid standards. Factors are reported as high versus low for each factor

characteristics of the method and its suitability to undergo multilaboratory testing for consideration as an official method.

The calibration curves generated for the individual curcuminoids throughout the optimization and validation studies were linear based on visual inspection. This was further confirmed by all curve having correlation coefficients greater than 0.997.

The dietary supplements used in this study were either single component or multicomponent products, where the other contents included boswellia, holy basil, rosemary, ginger, green tea, hu zhang, Chinese goldthread, barberry, black pepperfruit, oregano, and skullcap. There was no evidence of chromatographic interference at 425 nm by any of the other botanical sources used in the multicomponent products. The total curcuminoid contents in the capsules, tablets, and softgels ranged from 4.3 up to 882.1 mg/g, and tinctures ranged from 2.52 to 3.71 mg/mL. Raw materials ranged from 6.63 to 26.5 mg/g total curcuminoids. The individual curcuminoid contents are summarized in Table 4.

HorRat values were determined for the individual curcuminoids in all of the products, as summarized in Tables 4 and 5. These values were acceptable for all samples with the exception of the turmeric root sample 8019, as per the AOAC

Table 4 Precision results summary for turmeric-dried raw materials and dry finished products

Test sample ID number	Analyte	Mean (mg/g)	HorRat	RSD (%)
297	BDMC	<LOQ	n/a	n/a
	DMC	0.56	1.28	7.9
	Curcumin	3.77	1.51	7.0
298	BDMC	0.97	0.81	4.6
	DMC	6.15	1.20	5.2
	Curcumin	28.17	1.80	6.2
8019	BDMC	5.70	1.89	8.2
	DMC	5.06	1.96	8.7
	Curcumin	15.74	2.37	8.8
8020	BDMC	<LOQ	n/a	n/a
	DMC	1.46	1.13	6.0
	Curcumin	5.17	1.61	7.1
296	BDMC	33.46	0.80	2.7
	DMC	28.22	0.45	1.6
	Curcumin	76.06	0.29	0.9
300	BDMC	2.64	0.63	3.1
	DMC	3.59	0.60	2.8
	Curcumin	10.68	0.74	2.9
301	BDMC	ND	n/a	n/a
	DMC	ND	n/a	n/a
	Curcumin	882.10	1.42	2.9
293	BDMC	5.84	1.14	4.9
	DMC	45.03	1.02	3.2
	Curcumin	262.04	0.98	2.4
295	BDMC	3.83	1.42	6.6
	DMC	55.57	1.14	3.5
	Curcumin	390.76	1.35	3.1
4139	BDMC	9.43	1.25	5.0
	DMC	80.96	1.19	3.5
	Curcumin	659.15	1.62	3.4

<LOQ below limit of quantitation, ND not detected or below method detection limit, DMC demethoxycurcumin, BDMC bisdemethoxycurcumin, LOQ limit of quantitation

Fig. 4 Chromatographic separation of curcuminoid standards containing metanil yellow as an adulterant

Table 5 Precision results summary for turmeric liquid finished products

Test sample ID number	Analyte	Mean (µg/mL)	HorRat	RSD (%)
294	BDMC	537	0.49	3.0
	DMC	296	0.45	2.9
	Curcumin	661	0.53	3.2
299	BDMC	1164	0.33	1.8
	DMC	1040	0.33	1.8
	Curcumin	1501	0.40	2.1

DMC demethoxycurcumin, BDMC bisdemethoxycurcumin

International guidelines (AOAC International 2013). The acceptable HorRat values ranged from 0.29 to 1.96. The softgel sample 298 originally had significantly higher HorRat values than those reported in Table 4. Grubb's and modified z tests for outliers indicated that the curcumin value obtained from the second replicate on day 2 for this test sample was an outlier ($\alpha=0.075$). Removal of this outlier from the HorRat calculation provided the acceptable HorRat values. The total number of replicates, $n=11$ analyzed over three separate days was still acceptable as per the AOAC guidelines.

Inspection of the results for test sample 8019 revealed that results for the third and fourth replicates prepared on day 3 for this sample had lower analyte levels than the other replicates had for this sample. This suggests that these replicates were responsible for the poor observed precision. Grubb's and modified z tests, however, did not indicate the presence of any outliers in the sample. As the majority of the matrices were consistently within the recommended HorRat ranges, this one sample is likely due to sample preparation issues or sample inhomogeneity issues. Considering that this is one of the 12 samples tested in the validation and another raw material produced acceptable HorRats, the method is valid.

The calculated MDL and limits of quantification are shown in Table 6 for each of the curcuminoids. The calibration range of the curcuminoids was considerably higher in comparison with the quantitation limit, which restricted quantitation to within the calibration ranges used. Therefore, using the calibration curves described in the "Materials and Methods" section, the effective limit of quantification for bisdemethoxycurcumin, demethoxycurcumin, and curcumin were determined as 1.0, 1.0, and 5.0 µg/mL, respectively. Given the extraction

Table 6 Calculated method detection limit and limit of quantitation for the individual curcuminoid standards

Analyte	MDL (µg/mL)	LOQ (µg/mL)
BDMC	0.13	0.35
DMC	0.15	0.41
Curcumin	0.17	0.46

DMC demethoxycurcumin, BDMC bisdemethoxycurcumin

Table 7 Accuracy results for individual curcuminoids at three separate spike concentrations

Analyte	Concentration (µg/mL)	Mean recovery (%)
BDMC	5.0	102.3
	40.0	98.9
	75.0	96.6
DMC	5.0	101.8
	40.0	100.0
	75.0	97.7
Curcumin	5.0	103.3
	40.0	101.0
	75.0	98.5

DMC demethoxycurcumin, BDMC bisdemethoxycurcumin

protocol employed, these limits indicate that the method can quantify bisdemethoxycurcumin, demethoxycurcumin, and curcumin in solid samples at levels as low as 0.3, 0.3, and 1.6 mg/g, respectively. If the curcuminoid levels in a specific product are lower than the calibration range used in this study, the calibration range could be modified to as low as the limits of quantitation stated in Table 6.

qNMR was used to determine the purities of the curcuminoid standards. All three standards were reported to have very low levels of contaminants and greater than 98 % purity, therefore the purity provided in the suppliers C of A was used. The overall accuracy of the method was determined using spike recovery tests and ranged from 99.3 to 100.9 % for the individual curcuminoids. The recovery results are summarized in Table 7 and are considered acceptable according to the AOAC International guidelines.

Conclusions

Based on the factorial designs, the most important factors for curcuminoid quantitation in turmeric are column temperature and extraction solvent composition. These factors were modified to produce an optimized method which is suitable for quantitation of curcuminoids in turmeric raw materials and finished products. The method was then subjected to a single-laboratory validation according to the AOAC International guidelines. All of the parameters investigated were within the acceptable ranges described in the guidelines, with the exception of the precision for curcumin in one raw material, which is suspected to be attributed to sample preparation issues. The method was valid for the quantitation of all three curcuminoids in single and multicomponent products containing turmeric as an ingredient with HorRat values ranging from 0.29 to 1.80. For dried turmeric roots, the HorRat values ranged from 1.13 to 1.96; however, the determination of curcumin in turmeric root materials gave slightly higher than

the ideal HorRat value of 2.37, which is not thought to be an issue with the method. The overall accuracy of the method assessed at three separate levels for each analyte ranged from 99.3 to 100.9 % and was determined to be acceptable as per the AOAC guidelines. Despite the apparent poor precision observed for curcumin, one of the samples, which may be due to improper preparation of two of the replicates, this method is recommended for consideration as an official method or interlaboratory assessment.

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Compliance with Ethical Standards

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Conflict of Interest All authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human or animals subjects.

Informed Consent Not applicable.

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