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A Simple HPLC-fluorescence Method for Quantitation of Curcuminoids and Its Application to Turmeric Products

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An HPLC method using fluorescence detection for the quantitation of curcuminoids, such as curcumin (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) in turmeric products is described. This method involves a simple ultrasonic extraction with methanol as a pretreatment of turmeric products. The separation of curcuminoids and 2,5-xylenol (internal standard) was achieved within 30 min on a Cadenza CD-C_{18} column (250 × 4.6 mm; i.d., 3 μm) with a mixture of acetate buffer and CH_3CN. The calibration curves of standard curcuminoids showed good linearities of more than 0.993 of the correlation coefficient. The instrumental detection limits for C, DMC and BDMC (signal-to-noise ratio = 3) were 1.5, 0.9 and 0.09 ng mL^{-1}, respectively. The relative standard deviations of intra- and inter-day assays by curcuminoids spiked to turmeric powder were less than 6.1%. The proposed method was successfully applied to determine curcuminoids in commercial turmeric products, such as turmeric powders, a tablet, a dressing, a beverage, tea, and crude drugs.

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Introduction

Curcuminoids, such as curcumin [(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene] (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), are derived from the turmeric plant (*Curcuma longa* L.). These compounds are yellow pigments, which are responsible for the yellow color of the root of turmeric. These are commonly used as a dietary spice and a natural coloring agent in South and Southeast Asia. Over the years, curcumin was reported to have a wide range of pharmacological activities, including anti-inflammatory, anti-cancer, anti-oxide, anti-angiogenic and immunomodulatory.[1–3] Research has also shown that curcumin is a potential chemotherapeutic agent for cancer[4,5] as well as a promising agent in the treatment and prevention of Alzheimer's disease.[6,7] It is believed that these desirable effects may appear upon the intake of curcuminoids or turmeric products. Since the quality of turmeric products is directly based on the amount of the curcuminoids, a reliable method for the quantitative analysis of curcuminoids in turmeric products is required.

For the determination of curcuminoids, several HPLC methods have been described. In most cases, HPLC methods with UV/VIS detection at around 260 or 450 nm were used, since these methods require simple instruments, and are sufficiently sensitive to determine curcuminoids in rhizomes of *Curcuma* species,[8–10] turmeric products[11] and herbal medicinal preparations.[12] However, these methods are not selective, so a time-consuming pretreatment of a sample, or complicate gradient elution is required. The HPLC-MS method is highly sensitive and selective, but needs expensive instruments.[13,14]

On the other hand, since curcuminoids have native fluorescence, a more sensitive and selective quantitation method for curcumin derivatives compared to the UV/VIS detection method could be developed by using fluorescence (FL) detection. The FL detection method for curcumin and its structural isomers reported by Toennesen *et al.* was 10-times more sensitive than that of the UV detection method.[15]

In this study, HPLC-FL detection for the quantitation of curcuminoids in turmeric products was examined (Fig. 1). Furthermore, the proposed method was applied to determine curcuminoids in various forms (solid or liquid) of turmeric products.

Experimental

Chemicals and turmeric products

C, DMC and BDMC were purchased from Nacalai Tesque,

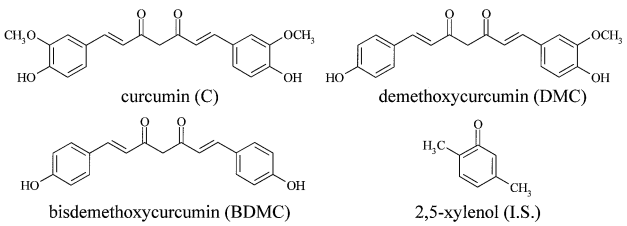

Fig. 1 Chemical structures of curcuminoids and I.S.

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Inc. (Kyoto, Japan). 2,5-Xylenol (internal standard, I.S.), sodium acetate and CH_3CN were purchased from Wako Pure Chemical Industries (Osaka, Japan). Acetic acid was obtained from Kishida Chemical Co. (Osaka). Other reagents used were of analytical reagent grade. Water was deionized and distilled by an Aquarius GSR-500 automatic water distillation apparatus (Advantec Mfs, Inc., Tokyo, Japan).

Methanolic solutions of C, DMC, BDMC at 0.5 mg mL^{-1} and 2,5-xylenol at 244 μg mL^{-1} were prepared as stock solutions. Working solutions of curcumin derivatives and I.S. were obtained by diluting the stock solution with methanol. These solutions were stored at 4°C between experiments.

Turmeric powders (A – I), a tablet (J), a dressing (K), a beverage (L) and tea samples (M – Q) were commercially available. Three kinds of crude drugs, such as *Curcuma longa* (R), *Curcuma aromatica* (S) and *Curcuma zedoaria* (T), were purchased from a market in China.

Extraction of curcumin derivatives in turmeric products

Turmeric powder and tablet: To 2.5 mg of a sample (powder or grinded tablet) and I.S., methanol was added up to 2.5 mL. The solution was vortex-mixed and then sonicated for 3 min. After filtration with a membrane filter (0.45 μm, Nacalai Tesque, Inc.), the solution was diluted with the mobile phase appropriately and applied to HPLC analysis.

Dressing containing turmeric powder or liquid samples: One gram of dressing or 400 μL of liquid samples was used for the determination of curcuminoids. After the addition of I.S., the sample was diluted with methanol up to 5 mL. The sample was sonicated for 5 min and centrifuged at 1000*g* for 5 min. Following filtration, the resulting solution was diluted 100 times (for dressing) or 20 times (for liquid sample) with the mobile phase, and applied to HPLC analysis. Tea drinks were analyzed by direct injection after filtration.

Crude drug: To 1 g of pulverized crude drug, 5 mL of methanol was added. After briefly vortex-mixing, it was soaked over night (for more than 12 h) and sonicated for 30 min. Then, the solution was centrifuged at 1000*g* for 5 min, and the supernatant was filtered (0.45 μm). The resultant solution was diluted with the mobile phase to obtain 20 mg mL^{-1} of *Curcuma longa*, 60 mg mL^{-1} of *Curcuma aromatica* and 20 mg mL^{-1} of *Curcuma zedoaria*.

HPLC system and conditions

An HPLC system for the determination of curcuminoids consisted of an LC-10AD_{VP} chromatographic pump (Shimadzu, Kyoto), a 7725 injector with a 100-μL sample loop (Rheodyne Inc., CA), a Cadenza CD-C_{18} column (250 × 4.6 mm; i.d., 3 μm, Imtakt Co., Kyoto), a CTO-10AS_{VP} column oven (Shimadzu), an RF-10A_{XL} fluorescence detector (Shimadzu), an R-01A recorder (Rikadenki Kogyo Co., Tokyo) and a UNI-1 noise cleaner (Union Co., Gunma, Japan). The column oven was set at 30°C. A mixture of 0.1 M of acetate buffer (pH 4.0) and CH_3CN (= 57:43, v/v) was used as a mobile phase and flowed at 1.0 mL min^{-1}. The detection wavelengths were set at 287 nm (λ_{ex}) and 303 nm (λ_{em}) for I.S. and 426 nm (λ_{ex}) and 539 nm (λ_{em}) for curcuminoids, respectively. The detection wavelength for I.S. was initially set and automatically changed to that for curcumin derivatives at 15 min after sample injection. The detection wavelengths of curcuminoids and I.S. were measured by a 650-10S fluorescence spectrophotometer (Hitachi, Ltd., Tokyo) using 10 μM of standard solutions.

Method validation

Instrumental and method detection limits of curcumin derivatives were defined as the concentration of the standard at a signal-to-noise (*S*/*N*) ratio of 3. For the quantification of curcuminoids, spiked calibration curves using turmeric products spiked with standard curcuminoids were prepared by the internal standard method. Calibration curves were constructed by plotting the concentration of curcuminoids *vs.* the peak height ratio of curcuminoids to I.S. However, curcuminoids in tea drinks and crude drugs were determined by an absolute calibration method.

The accuracy and precisions of intra- and inter-day assay precisions for the proposed method were examined by using turmeric powder spiked with known concentrations of curcumin standards (n = 5). The accuracy was represented as the ratio of the found concentration that corresponded to the spiked standard to the nominal concentration. The precision was represented as a relative standard deviation (RSD, n = 5). The recovery was calculated by using the peak height of curcumin standards spiked with turmeric powder and the corresponding standard. The presented recovery in the text was the mean of those spiked with the 2 concentrations of standards as well as an accuracy examination.

Results and Discussion

Extraction of curcumin derivatives in turmeric products

The time of sonication for the extraction of curcuminoids in solid turmeric products was examined. For turmeric powders or tablet, samples were sonicated for 1, 3, 5, 10, 15 and 25 min; however, the results showed no significant difference regarding the sonication times (data were not shown). Considering the prevention of an error, sonication for 3 min was chosen for the extraction of curcuminoids in turmeric powder or tablet. Next, for crude drugs, sonications for 3, 5, 10, 60 and 120 min with/without overnight soaking (for more than 12 h) were carried on. As a result, the concentration of curcuminoids extracted increased when longer sonication times without soaking were demonstrated, and the maximum and constant concentration of curcuminoids by sonicating for more than 30 min after overnight soaking was obtained (data were not shown). Therefore, 30 min of sonication after overnight of soaking was chosen for the extraction of curcuminoids in crude drugs.

HPLC separation

The detection wavelengths of curcuminoids and I.S. were measured by a fluorescence spectrophotometer using 10 μM of standard solutions in the mobile phase. The maximum wavelengths were 287 nm (λ_{ex}) and 303 nm (λ_{em}) for I.S. and 426 nm (λ_{ex}) and 539 nm (λ_{em}) for curcuminoids, respectively. The separation of curcuminoids was successfully achieved within 30 min by a Cadenza CD-C_{18} column (250 × 4.6 mm; i.d., 3 μm) with a mixture of 0.1 M of acetate buffer (pH 4.0) and CH_3CN (= 57:43, v/v) as a mobile phase. The retention times of I.S., BDMC, DMC and C were 11, 19, 22 and 25 min, respectively. In Fig. 2, typical chromatograms of a standard solution (a) and turmeric powder (b) are shown. The detection wavelengths initially set for I.S. were automatically changed to those for curcuminoids at 15 min after sample injection. The arrow in the chromatograms indicates changing the detection wavelengths. Three curcuminoids in turmeric products could be determined, though tailing of the BDMC peak in practical sample was observed by using this elution condition.

Method validation

Calibration curves of standard curcuminoids in the range of

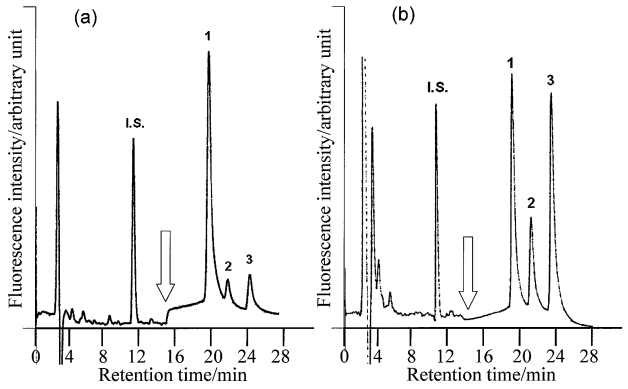

Fig. 2 Chromatograms of a standard solution (a) and turmeric powder G (b). The peak number: 1, BDMC; 2, DMC; 3, C. The arrow indicates changing of the detection wavelengths from 287 nm (λ_{ex}) and 303 nm (λ_{em}) for I.S. to 426 nm (λ_{ex}) and 539 nm (λ_{em}) for curcuminoids.

0.002 – 0.3 μg mL^{-1} for BDMC, 0.02 – 1 μg mL^{-1} for DMC and 0.03 – 3 μg mL^{-1} for C showed good linearities ($r \geqq 0.993$). The instrumental limits of detection (LOD) at an *S*/*N* ratio of 3 were 0.09 ng mL^{-1} for BDMC, 0.9 ng mL^{-1} for DMC, 1.5 ng mL^{-1} for C, respectively. The sensitivity of the proposed method for C was 10-times higher than that of a method reported previously.[15] For the quantitation of curcuminoids, spiked calibration curves were prepared. The calibration range for turmeric powder and dressing were 0.4 – 20 mg g^{-1} (BDMC), 0.5 – 25 mg g^{-1} (DMC) and 2 – 80 mg g^{-1} (C), and 0.015 – 0.3 mg g^{-1} (BDMC), 0.05 – 1 mg g^{-1} (DMC) and 0.15 – 3 mg g^{-1} (C), respectively. The method LODs in turmeric powder at a *S*/*N* ratio of 3 were 0.09 μg g^{-1} for BDMC, 1.0 μg g^{-1} for DMC and 3.4 μg g^{-1} for C, respectively. For the determination of curcuminoids in tea drinks, absolute calibration curves by using tea-spiked curcuminoid standards were prepared in the range of 2×10^{-6} – 7×10^{-4} mg g^{-1} for BDMC, 4×10^{-6} – 5×10^{-4} mg g^{-1} for DMC and 5×10^{-5} – 8×10^{-4} mg g^{-1} for C. The recoveries of curcuminoids from turmeric powder were examined by using the peak heights of standards and those spiked in turmeric powder. The recoveries for C, DMC and BDMC were 102.2, 98.4 and 96.4%, respectively.

The accuracy, intra- and inter-day assay precision for curcuminoids were studied by turmeric powder spiked with known concentrations of standards (Table 1). The accuracy ranged from 94.1 to 104.7%. The precisions (RSD) of intra- and inter-day assays were less than 5.2 and 6.1%, respectively. These results indicate that the proposed method is well-validated and suitable to determine curcuminoids in turmeric products.

Yang *et al.* have reported a LC-MS/MS method for the determination of DMC and C in *Curcuma longa*.[16] The LOD was 1 ng ml^{-1} and the precision was within 19%. Compared to their method, though our method did not improve the LOD, the precision was lower, 6.1%. Also, an HPLC-FL requires simple and inexpensive instruments, and is more convenient than LC-MS/MS. The proposed method is the first HPLC-FL to determine BDMC, DMC and C simultaneously, and can be applied to not only herb material, but also to various turmeric products.

Determination of curcumin derivatives in turmeric products

The proposed method was applied to determine curcuminoids in commercial turmeric products, such as turmeric powders, a tablet, a dressing, a beverage, tea, and crude drugs. The results of 20 samples are summarized in Table 2. The amounts of curcuminoids in nine turmeric powders (A – I), which were produced by different makers, showed a wide range. Curcuminoids in sample C were remarkably high, since this sample was extracted turmeric powder. In sample K, 0.15 mg of BDMC, 0.16 mg of DMC and 0.50 mg of C were found in 1 g of dressing. These amounts of curcuminoids agreed with those in turmeric powder used as the raw material of dressing. As for sample L, a turmeric beverage, the curcumin content (29.5 mg 100 mL^{-1}) agreed well to that indicated on the label of the bottle (30 mg 100 mL^{-1}). In tea drinks (M – Q), the amount of curcuminoids varied over a wide range according to the makers. Crude drugs (R – T) included small amounts of curcuminoids.

Table 1 Accuracy, intra- and inter-day precision of the proposed method

Compound	Added/ μg mg^{-1}	Found/ μg mg^{-1}	Accuracy, %[a]	Precision (RSD, %)[a]	
				Intra-day	Inter-day
BDMC	—	2.69	—	4.2	5.1
	1.25	3.99	103.7	2.3	4.4
	5.00	7.93	104.7	3.4	4.3
DMC	—	3.61	—	5.2	4.7
	1.75	5.10	99.3	2.4	4.6
	6.25	9.50	94.1	2.9	4.4
C	—	10.38	—	5.0	4.7
	3.75	14.19	101.6	3.4	6.1
	15.00	25.68	102.0	4.0	4.2

a. $n = 5$.

Though the amounts of the turmeric powder (A – I) made by different makers were quite different, they show a similar content composition (Fig. 3).

In tea drinks (M – Q), the composition of curcumin was quite low compared with other products. Curcumin has been reported to be unstable under neutral-basic conditions. About 90% of the compound decomposed within 30 min in 0.1 M phosphate buffer at pH 7.2.[17] Moreover, curcumin is known to have poor solubility in water.[18] Therefore, it may be difficult to obtain a high concentration of curcumin in aqueous tea of a neutral-base.

The composition of curcuminoids in *Curcuma longa* (R), *Curcuma aromatica* (S) and *Curcuma zedoaria* (T) were quite different, as shown in Fig. 3. These characteristics may help to distinguish three species from each other.

Conclusion

A simple HPLC-FL method was developed for the analysis of three kinds of curcuminoids, such as BDMC, DMC and C, in turmeric products. This method was sensitive and well-validated to determine curcuminoids in turmeric products. Furthermore, application data to various forms of turmeric products suggested that the method was powerful for the analysis of turmeric products.

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Table 2 Concentrations of curcuminoids in turmeric products

Turmeric product		Concentration (mean ± S.D.[a])/mg g^{-1}		
		BDMC	DMC	C
A	Powder	1.73 ± 0.03	2.62 ± 0.08	7.57 ± 0.11
B	Powder	1.63 ± 0.01	2.23 ± 0.04	6.64 ± 0.04
C	Powder	19.54 ± 0.31	21.79 ± 0.80	69.21 ± 0.08
D	Powder	2.99 ± 0.07	2.67 ± 0.02	8.01 ± 0.17
E	Powder	3.80 ± 0.05	4.11 ± 0.02	12.75 ± 0.21
F	Powder	2.78 ± 0.09	3.68 ± 0.17	10.69 ± 0.25
G	Powder	2.50 ± 0.04	3.77 ± 0.09	10.43 ± 0.23
H	Powder	1.31 ± 0.09	1.31 ± 0.08	3.97 ± 0.20
I	Pigment powder	2.64 ± 0.12	1.69 ± 0.06	4.54 ± 0.08
J	Tablet	2.02 ± 0.10	3.50 ± 0.19	12.62 ± 0.50
K	Dressing	0.15 ± 0.01	0.16 ± 0.01	0.50 ± 0.02
L	Beverage	(2.00 ± 0.05)× 10^{-2}	(9.36 ± 0.24)× 10^{-2}	0.29 ± 0.01
M	Tea drink	(6.41 ± 0.19)× 10^{-4}	(3.58 ± 0.14)× 10^{-4}	(6.52 ± 0.20)× 10^{-4}
N	Tea drink	(3.00 ± 0.03)× 10^{-4}	(9.24 ± 0.00)× 10^{-5}	(7.98 ± 0.49)× 10^{-5}
O	Tea drink	(2.61 ± 0.11)× 10^{-5}	(1.13 ± 0.09)× 10^{-5}	Trace[b]
P	Tea drink	(7.12 ± 0.30)× 10^{-5}	(2.57 ± 0.18)× 10^{-5}	Trace[b]
Q	Tea drink	(4.76 ± 0.64)× 10^{-6}	(1.99 ± 0.22)× 10^{-6}	N.D.[c]
R	*Curcuma longa*	(2.68 ± 0.34)× 10^{-4}	(9.57 ± 0.81)× 10^{-4}	(5.36 ± 0.63)× 10^{-4}
S	*Curcuma aromatica*	Trace[b]	(2.72 ± 0.21)× 10^{-5}	(7.35 ± 1.05)× 10^{-5}
T	*Curcuma zedoaria*	(3.24 ± 0.37)× 10^{-3}	(7.89 ± 0.93)× 10^{-2}	(5.70 ± 0.70)× 10^{-2}

a. n = 3. b. Trace, lower than limit of quantitation but higher than LOD. c. N.D., not detected.

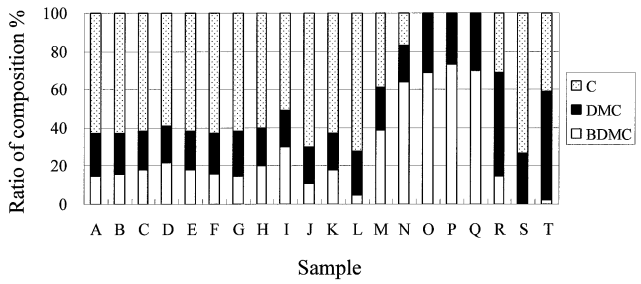

Fig. 3 Composition ratio of curcuminoids in the turmeric products.

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