Quantification of Bisacurone and Curcuminoids in Turmeric Products by Liquid Chromatography Coupled with Tandem Mass Spectrometry

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Summary Turmeric products have many useful physiological functions and are widely used as health food and food ingredient. Here, we report the use of HPLC-ESI-MS/MS to simultaneously quantify bisacurone and three curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) in turmeric products (high viscosity liquid, granular powder, tablet, and solution). The results showed that the standard values and measured values of curcumin in each product were almost same. Demethoxycurcumin and bisdemethoxycurcumin were contained in each products. Meanwhile, the content of bisacurone differed greatly among the products. In particular, the highest amount of bisacurone was found in the turmeric product A (high viscosity liquid, 9.48 g/100 g product). It would become important to consider the bisacurone content in turmeric products.

Key Words bisacurone, curcumin, plant derived food, tandem mass spectrometry, turmeric

Turmeric (Curcuma longa L.) has various beneficial effects for human health benefits, such as anti-angiogenic, anti-bacterial, anti-fungal, anti-obesity, anti-oxidant, anti-inflammatory, anti-metastatic, anti-mutagenic, anti-viral, immunomodulatory, neuroprotection, and wound healing activities (1, 2). Therefore, the usefulness of using turmeric for the prevention and treatment of various diseases and the promotion of health has been widely studied around the world. Content of various components in turmeric varies depending on the various factors, such as variety, location, growing conditions (3). Even in the manufacturing process of products using turmeric, extraction and storage processes also have a significant impact on its content (4). Because of this, the variability in the quality of turmeric and its products, as well as content of functional ingredients in them have been pointed out and raised concerns (5). In products made from turmeric, three curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) are considered as the main quality control compounds (Fig. 1) (6). Therefore, measurement of these three curcuminoids are important for product development and quality control.

On the other hand, among the functional components other than curcuminoids in turmeric, bisacurone have been attracting much attention (Fig. 1). Compared to curcuminoids, bisacurone has recently been found to have the potential to exert stronger physiological effects (7). For example, Megumi et al. observed the protective effects of curcumin and bisacurone against ethanol-induced liver injury by a rat primary hepatocyte model (8). As a result, they reported that only bisacurone showed a predominant hepatoprotective effect at a low concentration (1 μM). Despite the growing interest in such studies, the analytical procedures for the detection of bisacurone have received comparatively little attention. Therefore, it is essential to quantify the curcuminoids and bisacurone in turmeric products and evaluate their efficacy.

In general, concentration of bisacurone displayed in ingredient labels of turmeric products are based on quantitative data using the liquid chromatography coupled with ultraviolet detection (HPLC-UV) method. Two previous reports quantified bisacurone in turmeric extracts by HPLC-UV, and both reported that bisacurone was separated in a reversed-phase column and could be measured at wavelengths of 240 or 245 nm (9, 10). However, there is no report on the quantification of bisacurone by liquid chromatography coupled with tandem mass spectrometry (MS/MS), except for one report on the quantification of its concentration in rhizome of turmeric (11). Liquid chromatography coupled with electrospray ionization tandem mass spectrometry

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(HPLC-ESI-MS/MS) method is considered to be an accurate and reliable tool in the quantification of selected compounds. Therefore, in this study, we determined bisacurone and curcuminoids in several turmeric products simultaneously by HPLC-ESI-MS/MS to compare their amounts.

**MATERIALS AND METHODS**

Materials and methods in this study are available in “Materials and Methods” section of Supporting Information and Fig. S1 (Supplemental Online Material).

**RESULTS**

Each analytical standard (bisacurone, curcumin, demethoxycurcumin, and bisdemethoxycurcumin) with 500 ng/mL were optimized by MS/MS system via infusion. Mass spectrometer was operated in the positive ionization mode and bisacurone was detected to have a form in which two water molecules were eliminated (\([M-2H_2O]^+\), Fig. S2 A (Supplemental Online Material)). On the other hand, curcumin, demethoxycurcumin, and bisdemethoxycurcumin were detected to have a form in which hydrogen adduct (\([M+H]^+\), Fig. S2 B–D (Supplemental Online Material)), which are commonly observed by the ESI. Precursor ions to the product ions (Q1>Q3) were chosen for multiple reaction monitoring (MRM) detections of each compound. MRM for each compound were as follows: bisacurone, (217>119); curcumin, (369>145); demethoxycurcumin, (339>147); bisdemethoxycurcumin, (309>147) (Fig. S2 A–D (Supplemental Online Material)). Other parameters (desolvation potential [DP], entrance potential [EP], collision energies [CE] and collision cell exit potential [CXP]) were also optimized and each condition are summarized in Table S1 (Supplemental Online Material).

Then, the optimization of the ion source of HPLC-ESI-MS/MS using an analytical standard mixture of 100 ng/mL resulted as follows: ionization, ESI (positive); ion source, turbo spray; collision gas (CAD), \(N_2\) (5 psi); curtain gas (CUR), \(N_2\) (10 psi); ion source gas 1 (GS1), air (40 psi); ion source gas 2 (GS2), air (50 psi); ionspray voltage (IS), 5,500 V; temperature (TEM), 500˚C; channel electron multiplier (CEM), 5,500 V. Constructed HPLC conditions for the separation of each standards were follows. Column was a YMC-Pack Pro C18 and the column eluent was binary gradient consisting of the solvent A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient profile was as follows: 0–0.5 min, 10% B; 0.5–12 min, 80% B; 12–15 min, 10% B. The flow rate was adjusted to 0.3 mL/min, injection volume was 5 \(\mu\)L, and the column temperature was maintained at 40˚C. Under the above HPLC-ESI-MS/MS conditions in this study, the chromatograms of each standard were obtained as shown in Fig. S3 A–D (Supplemental Online Material). With this program, each compound was separated in 15 min. Then, standard solutions of 10, 20, 50, 100, 200, 300, 400, and 500 ng/mL were prepared and analyzed under this HPLC-ESI-MS/MS conditions, and the generated standard curves (\(r>0.997\)) are shown in Fig. S4 A–D (Supplemental Online Material). As a result, it was confirmed that quantification was possible in the concentration range of 10 to 500 ng/mL.

Content of bisacurone, curcumin, demethoxycurcumin, and bisdemethoxycurcumin in each turmeric product (A, B, C, D and E) were measured by HPLC-ESI-MS/MS method in this study. The chromatograms of each turmeric products were obtained as shown in Fig. 1. Chemical structures of the bisacurone and curcuminoids in turmeric products, for which quantitative methods were investigated in this study.

**Fig. 1.** Chemical structures of the bisacurone and curcuminoids in turmeric products, for which quantitative methods were investigated in this study.

| Name of product | Bisacurone | Curcumin | Demethoxycurcumin | Bisdemethoxycurcumin |
|-----------------|------------|----------|--------------------|----------------------|
| A (high viscosity liquid) | 8.00 g/100 g | 9.48 g/100 g | 1.69 g/100 g | 1.01 g/100 g |
| B (granular powder) | 0.026 g/100 g | 0.030 g/100 g | 2.00 g/100 g | 2.40 g/100 g |
| C (tablet) | — | Not detected | 10.00 g/100 g | 10.46 g/100 g |
| D (solution) | 0.400 g/100 mL | 0.824 mg/100 mL | 30 mg/100 mL | 35 mg/100 mL |
| E (solution) | — | Not detected | 60 mg/100 mL | 62 mg/100 mL |

"Specified value" was referred to the ingredient labeling of each products. The values measured by LC-MS/MS in this study were shown as "Measured value."
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Disclosure of state of COI and wrote the manuscript. Teruo M. and O.H. Authorship stability needs to be evaluated in the future. Although there are no reports yet, stability of bisa-confirmation of its content is expected to become necessary. The quantitative results of each turmeric product were shown in Table 1. The content of bisacurone in turmeric products varied greatly from product to product. Measured values of bisacurone were almost the same as those of turmeric products that have a standard value of bisacurone in ingredient labeling. However, there were turmeric products in which bisacurone was not detected at all by HPLC-ESI-MS/MS. Specified and measured values of curcumin in each product were almost same. On the other hand, the ingredient labels of the turmeric products used in this experiment did not indicate the content of demethoxycurcumin and bisdemethoxycurcumin, so we could not compare the specified values with the actual measured values, but we confirmed that all the products contained them.

**DISCUSSION**

In this study, various forms (high viscosity liquid, granular powder, tablet, solution) of turmeric products were used as sample analysis, and all forms could be analyzed by HPLC-ESI-MS/MS by processing with the extraction method established in this study. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin were detected in all of the turmeric products measured in this study. On the other hand, the ingredient labels of the turmeric products in which bisacurone was not detected at all by HPLC-ESI-MS/MS. Specified and measured values of curcumin in each product were almost same. On the other hand, the ingredient labels of the turmeric products used in this experiment did not indicate the content of demethoxycurcumin and bisdemethoxycurcumin, so we could not compare the specified values with the actual measured values, but we confirmed that all the products contained them.

**Authorship**

Taiki M., O.H., M.S., and M.T.N.T. conducted experiments and wrote the manuscript. Teruo M. and O.H. designed the experiments.

**Disclosure of state of COI**

The authors report no conflicts of interest in this work.

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**Supporting information**

Supplemental online material is available on J-STAGE.

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