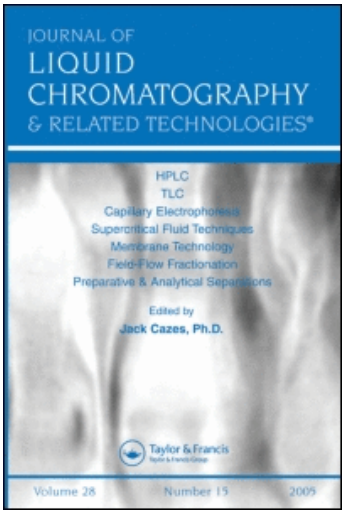

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Validation of LC/Electrospray-MS for Determination of Major Curcuminoids in Foods

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ABSTRACT

The aim of the present study is to establish an LC/MS method for the determination of major curcuminoids in foods. The LC/MS that was an electrospray ionization interface is employed for the evaluation of curcuminoids obtained by solid-phase extraction from foods. In addition, by determining the three kinds of curcuminoids quantitatively per gram of turmeric, it is possible to determine the constant used in calculating the

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content of turmeric in foods. The detection limit (DL) and the linearity of the calibration curve are calculated using the curcumin standard. DL is 1.0 ng/mL and the linearity of the calibration curve for curcumin has correlation coefficients exceeding 0.999. Therefore, the present method may be used in the routine determination of curcuminoids in foods.

Key Words: Curcuminoids; LC/Electrospray-MS; Solid-phase extraction.

INTRODUCTION

The rhizomes of *Curcuma longa* Linn (turmeric) are widely used as natural coloring agents in many foods. The major coloring substances in turmeric are curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] and two related demethoxy compounds (Fig. 1). These three major yellow pigments belong to the group of diarylheptane derivatives. Recently, these compounds were found to have antispasmodic, anticoagulant, and antitumor activities.[1–3] Thus, turmeric has been widely used as a food additive, condiment, and health food. A variety of methods for the determination of these compounds have been published.

Turmeric samples were analyzed to identify the major components using GC/MS and LC/MS.[4–6] Those studies revealed that the major components of turmeric are curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. The colored complex can be measured quantitatively by the reaction with boric acid.[7,8] Other methods for the quantification of total turmeric compounds include a direct spectrophotometric method[9] and a direct fluorometric method.[10] In addition, rapid measurement of curcuminoids was achieved by flow-injection analysis with fluorometric detection.[11] Moreover, the

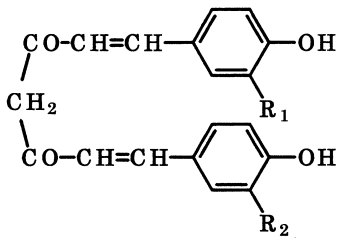

Figure 1. Structural formulae of turmeric curcuminoids. Curcumin: $R_1 = R_2 = OCH_3$ (MW = 368). Demethoxycurcumin: $R_1 = H$, $R_2 = OCH_3$ (MW = 338). Bisdemethoxycurcumin: $R_1 = R_2 = H$ (MW = 308).

determination of curcuminoids was reported.[12] Recently, GC, or HPLC with MS, was used to characterize the curcuminoids.[4–6] However, very few methods have been reported for the residual evaluation of curcuminoids in foods.[13] Therefore, the purpose of this study is to evaluate the curcuminoids, curcumin, desmethoxycurcumin, and bisdemethoxycurcumin, in foods by an accurate and sensitive LC/MS method.

EXPERIMENTAL

Reagents and Samples

Solvents of HPLC grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Water purified by the Milli-Q water-purification system (Millipore, Bedford, MA, USA) was used. The turmeric pigment was a present from San-Ei Gen F.F.I., Inc. (Osaka, Japan). Curcumin standard was purchased from Wako Pure Chemical Industries, Ltd..

Concentrated solutions (1.0 mg/mL) of curcumin and turmeric were prepared in methanol, and aliquots (0.5–5000 ng/mL) were prepared as required by the addition of methanol.

Tablet, tea, and candy samples were obtained randomly at various supermarkets and convenience stores in Japan.

Apparatus and Instrument Conditions

LC/MS was performed using an Agilent 1100 MSD-SL system (Agilent Technologies, Palo Alto, USA). A photodiode-array detector was set at 250 nm. Senshu Pak PEGASIL ODS (2 × 150 mm, 5 μm) reversed-phase column (Senshu Scientific Co., Ltd., Tokyo, Japan) was used. Samples of 5.0 μL volume were injected. LC separation was carried out using mobile phases of 0.01% acetic acid in water (mobile phase A) and acetonitrile (mobile phase B). The gradient mode was as follows: 0 min at 45% mobile phase B, then 0–15 min with a linear increase from 45 to 95% mobile phase B, and finally holding at 95% mobile phase B. The flow rate was 0.2 mL/min. The working conditions for electrospray ionization MS were as follows: the drying nitrogen gas temperature was set at 350°C and the gas was introduced into the capillary region at a flow rate of 12 L/min; the capillary was held at a potential of 3500 V relative to the counter electrode in the negative-ion mode. The fragmentor voltage was fixed at 120 V for curcumin and curcuminoids during the chromatographic run. When working in the selected ion monitoring (SIM) mode, ions at m/z 307, 337, and 367, which were assigned to $[M-H]^-$ of

bisdemethoxycurcumin, desmethoxycurcumin, and curcumin, respectively, were monitored.

Sample Preparation

The extraction of curcuminoids was carried out by using methanol. Curcuminoids were extracted from 0.1 g of tablets by adding 10 mL of methanol and ultrasonication for 10 min (ULTRA sonic 104 × 340 W, Yucaipa, California, USA). The sample was re-extracted with methanol in the same way three times. A 0.1 mL aliquot of the methanol extract was added into a tube, and then it was evaporated to dryness under a stream of nitrogen at 40°C. The sample was reconstituted with 500 mL of methanol.

In the case of samples with low curcuminoids content, we carried out solid-phase extraction (SPE) as the pretreatment process. The SPE cartridge for the pretreatment of curcuminoids in samples was BOND ELUT C_8 size: 500 mg/3 mL, (Varian Co., USA). The curcuminoids in food samples were pretreated using SPE cartridges of C_8-based phase. The solid samples (table and candy) were dissolved by ultrasonation in 3 mL of distilled water. The tea samples were directly extracted as follows. Before extracting the samples, the SPE cartridge was conditioned by washing 3.0 mL of methanol followed by 3.0 mL of distilled water. Ten milliliters of the liquid sample was eluted through the SPE cartridge. Then, this SPE cartridge was washed with 3.0 mL of water. Elution of 5.0 mL of methanol at a low flow rate was carried out to elute the compounds that were retained on SPE cartridge. The collected fraction was evaporated to dryness under a stream of nitrogen at 40°C. Then, the residue was dissolved in 1.0 mL of methanol. When a sample showed a concentration exceeding the calibration curve, a larger volume of methanol was used for reconstituting the sample. The obtained samples were measured by LC/MS.

RESULTS AND DISCUSSION

LC/MS Detection of Curcuminoids

In the spectral investigation by LC/MS in the SCAN mode of turmeric solutions (10 μg/mL), ions at m/z 307, 337, and 367, which were assigned to the $[M–H]^-$ ions of curcuminoids, were observed as the main peaks (Fig. 2).

The most important parameters affecting the determination of the compounds by LC/MS are the fragmentor voltage and concentration of acetic acid in water in the mobile phase. In order to establish the optimum fragmentor voltage for the detection of curcumin, the signal at m/z 367 was measured at

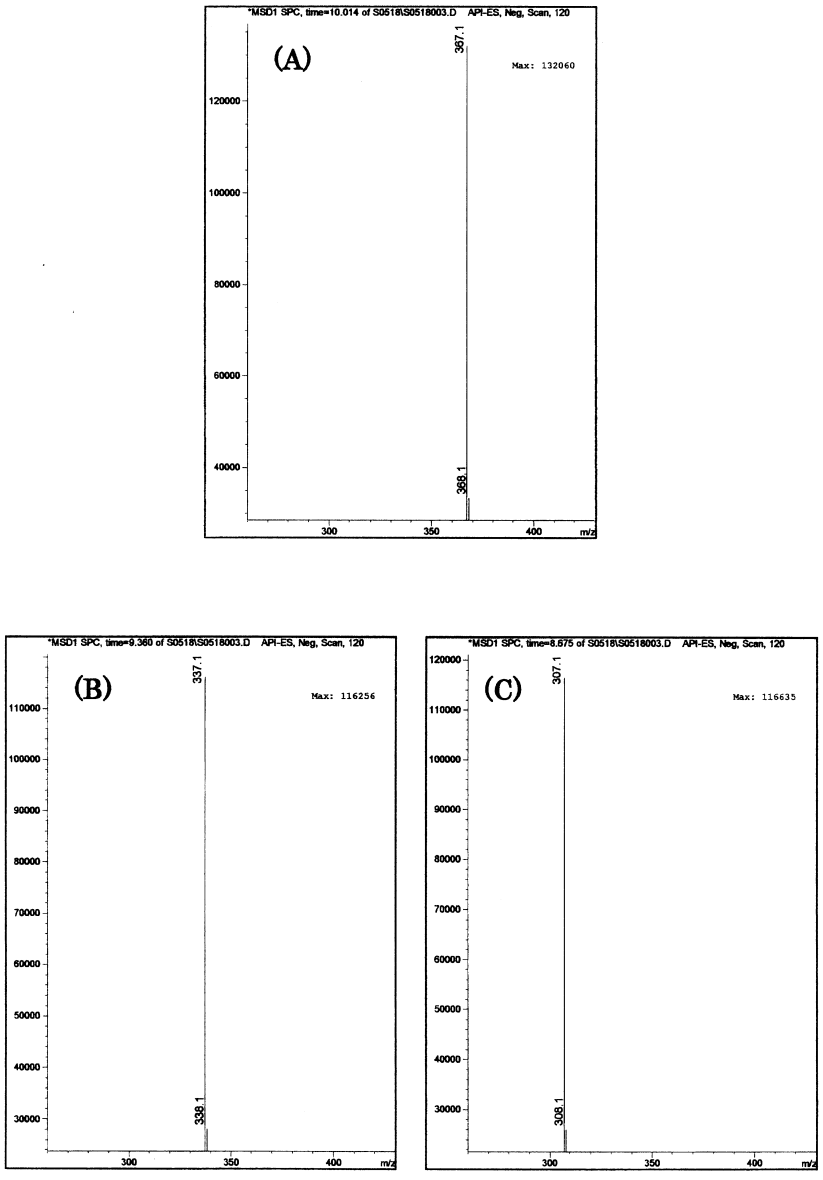

Figure 2. LC/MS-ESI spectra of curcumin (A), demethoxycurcumin (B) and bisdemethoxycurcumin (C) at fragmentor voltage of 120 V.

various fragmentor voltages. This resulted in an optimum fragmentor voltage of 120 V. Therefore, when the fragmentor voltage was set at 120 V, standard solutions of curcumin were infused with various concentrations of the mobile phase (water/acetic acid/acetonitrile) into the electrospray interface while the fragmentor voltage was varied. The signal intensity was a maximum at 0.01% acetic acid at 120 V for the detection of curcumin (Fig. 3). Therefore, this condition was used for the detection of curcuminoids.

Chromatographic Conditions and Evaluation Method of the Calculated Value

The detection limits (DLs) were calculated according to $3S_b = A_s - A_b$ (A_s is the average of the sample signal, A_b is the average of the blank signal, and S_b is the standard deviation of the blank signal). The DL of curcumin standard solution was 1.0 ng/mL. A sensitive and selective analysis method was realized using the LC/MS method for the determination of curcumin.

In this study, the quantitative determination of turmeric in foods was achieved by mean of LC/MS detection of these three kinds of curcuminoids, namely, curcumin, bisdemethoxycurcumin, and demethoxycurcumin. When turmeric powder was measured by LC/MS-SIM, chromatograms of curcumin, bisdemethoxycurcumin, and desmethoxycurcumin, were respectively observed. For the purpose of this study, the calculation of the mean values of these three major compounds was useful. Therefore, we quantitatively

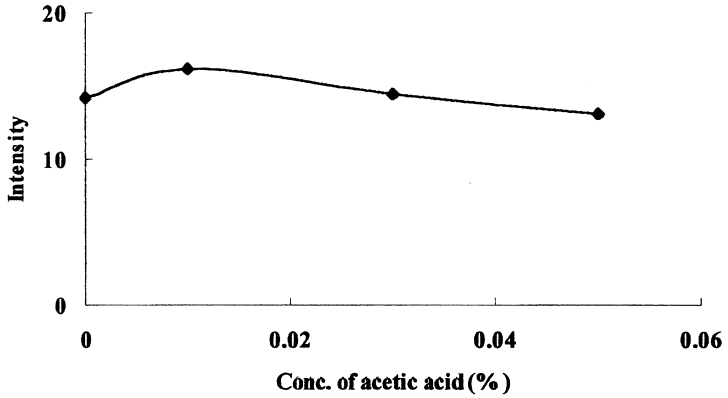

Figure 3. Effect of the composition of the mobile phase (water/acetic acid/acetonitrile) on the intensity of the $[M-H]^-$ ion of curcumin.

determined turmeric in foods using individual calibration curves (linearity of these compounds within 2.5–250 ng of turmeric powder). The following results were obtained: curcumin ($r = 0.9996$, $Y = 61.5X + 18130$), bisdemethoxycurcumin ($r = 0.9998$, $Y = 118.2X - 4331$), and demethoxycurcumin ($r = 0.9997$, $Y = 36.8X - 3154$). The analytical performance of the above was found to be reliable, sensitive, and suitable for routine analysis. It was possible to separate and determine these compounds in a single run of 15 min by using LC/MS-SIM. The method enables the precise determination of standards and may be used in detecting trace amounts of turmeric in food samples.

Sample Preparation

The SPE with the C_8-based phase was examined in terms of recovery, relative standard deviation (RSD), and clean-up effect. The recovery of the three curcuminoids was performed using distilled water spiked with a solution of turmeric at concentrations of 1.0 and 10 μg/mL. The extraction using the SPE cartridge was performed according to the above-described method. The percentage recovery of curcuminoids was above 85% with the C_8 phase (Table 1). Therefore, the SPE method is applicable s to food samples. Figure 4 shows the chromatograms obtained from the food samples. It is obvious that the C_8-phase SPE has good selectivity. Thus, we decided to use the C_8 cartridge for the simple and selective pretreatment of curcuminoids in food samples.

Application

In this study, a total of eleven samples comprising tablets, tea, and candies were analyzed by the present method. The curcuminoids, curcumin, bisdemethoxycurcumin, and demethoxycurcumin, were detected from all samples.

Table 1. The recovery of curcuminoids from distilled water.

	Recovery (%)		RSD (%) (n = 6)	
	10 μg/g	1 μg/g	10 μg/g	1 μg/g
Curcumin	94.6	92.9	3.4	5.6
Bisdemethoxycurcumin	91.6	86.1	3.6	7.9
Demethoxycurcumin	96.5	85.8	2.3	7.4

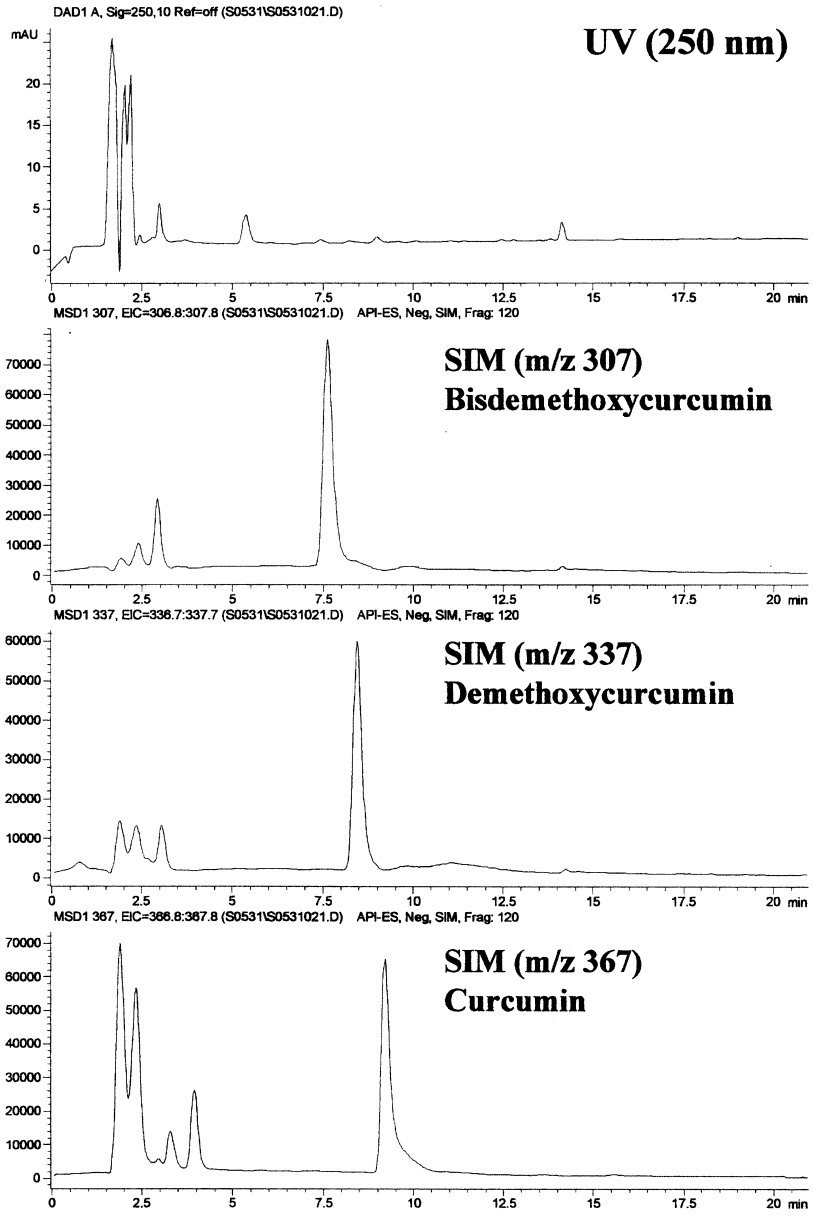

Figure 4. LC/MS SIM chromatograms of food sample (No. 8) in which turmeric was detected.

Table 2. Residual turmeric in foods.

		Turmeric (μg/g)			
		Curcumin	Demethoxycurcumin	Bisdemethoxycurcumin	
Number	Sample	*n*	*n*	*n*	Average
1	Tablet	106292.3	108461.5	111923.1	108892.3
2	Tablet	164750.0	147750.0	36250.0	116250.0
3	Tablet	581006.3	372656.3	96665.6	350109.4
4	Tea	110.4	125.6	79.7	105.2
5	Tea	1802.9	2841.3	3914.2	2852.8
6[a]	Tea	24.8	43.1	258.9	108.9
7	Candy	1.2	2.7	4.5	2.8
8	Candy	3.9	6.1	5.7	5.2
9	Candy	113.4	130.6	150.8	131.6
10	Candy	43.1	58.0	59.7	53.6
11	Candy	1.3	1.7	2.0	1.6

[a]Only the unit of sample No. 6 is in ng/mL.

The average amounts of curcuminoids ranged from 108.9 ng/g to 350.1 mg/g. The results are shown in Table 2. The detection levels of turmeric in foods were very low and residual curcuminoids could not be detected by LC/UV (250 nm). However, use of LC/MS-SIM and SPE enabled the successful determination of trace amounts of curcuminoids in food samples (Fig. 4).

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