Liquid Chromatographic Techniques in Food Sciences

Regular Article

Study on the Synthesis of Methylated Reference and Their Application in the Quantity of Curcuminoids Using Single Reference Liquid Chromatography Based on Relative Molar Sensitivity

Miki Takahashi,^a Koji Morimoto,^a Yuzo Nishizaki,^b Naoko Masumoto,^b Naoki Sugimoto,^b
Kyoko Sato,^b and Koichi Inoue*,^a

^a College of Pharmaceutical Sciences, Ritsumeikan University; 1–1–1 Nojihigashi, Kusatsu, Shiga 525–8577, Japan; and ^b National Institute of Health Sciences; 3–25–26 Tonomachi, Kawasaki-ku, Kawasaki 210–9501, Japan.

Received July 26, 2021; accepted September 7, 2021

We report on the recommendation of the simple and versatility of methylated reference (MR) to improve applications in the single reference (SR)-LC based on relative molar sensitivity (RMS). Three curcuminoids (Curs) such as curcumin, demethoxycurcumin and bisdemethoxycurcumin in turmeric products were determined using authentic standards and methylated curcumin. In addition, high-speed countercurrent chromatography (HSCCC) purification is necessary to separate Curs for indicating the RMS. For HSCCC separation, a biphasic solvent system was used to obtain these fractions, which were then subjected to ¹H quantitative NMR to determine their contents in each test solution. Using these solutions, the RMS of Curs are calculated from slopes ratios of calibration curves (three ranges from 0–100 μmol/L, $r^2 > 0.998$). The averaged RMS of Curs were 8.92 (relative standard deviation (RSD), 1.17%), 8.97 (2.18%), and 9.61 (0.77%), respectively. Cur concentrations in turmeric products can be determined using RMS, peak area, and MR content added in these samples. This proposed method, which is based on chemical methylation and the SR-LC assay has been successfully applied for the simple and reliable estimation of Curs in turmeric products.

Key words curcumin; methylation; relative molar sensitivity; methylated reference; high-speed countercurrent chromatography

Introduction

Curcumin (Cur 1) is a polyphenolic compound and isolated from turmeric (*Curcuma longa*) rhizome that also contains demethoxycurcumin (Cur 2) and bisdemethoxycurcumin (Cur 3) (Fig. 1-(a)). Three curcuminoids (Curs) such as Cur 1, Cur 2 and Cur 3 in turmeric products are well-known for their anti-inflammatory, antioxidant, and immunomodulatory effects,¹⁾ and recently interesting approach of its supplementation for declared coronavirus disease 2019 (COVID-19).^{2–4)} Based on chemical antioxidant properties, it has a wide range of pharmacological preservation for infection, cancer, nerve disease and aging. Thus, directions for future investigations to be undertaken in the chemistry of Curs have been proposed.⁵⁾ Indeed, the chemical antioxidant of turmeric powder showed the different effect of origin and isolated Curs.⁶⁾ A recent frame of adequacy indicated the comparative bioavailability of each Curs was investigated for pharmacokinetic study.⁷⁾ On the other hand, for analytical chemistry, many methods were reported for the quality assessment of Curs in biological and pharmaceutical matrices.⁸⁾ The evaluation of Curs from turmeric products would be needed for quality assessment in widespread use. On the other hand, turmeric extract on the market is usually a mixture of Curs. Thus, it is the first step to isolate and purify Curs (respective Cur 1, Cur 2, and Cur

3) for procuring these certified references, which is important using chromatographic techniques, such as centrifugal partition chromatography,⁹⁾ supercritical fluid chromatography,¹⁰⁾ and high-speed countercurrent chromatography (HSCCC).^{11,12)} Subsequently, a LC assay is the mainstream method for the quantity of Curs in various turmeric samples.¹³⁾

Single reference (SR)-LC assay based on relative molar sensitivity (RMS) is a recently applied method for the quantitative determination of natural compounds in various food materials.^{14–17)} Each natural analyte could be quantitated according its intensity ratio with SR coefficient on a one-running chromatogram.¹⁸⁾ Compare to conventional LC evaluation, SR-LC assay demonstrates that the calibration analysis of RMS using ¹H-quantitative nuclear magnetic resonance spectroscopy (qNMR) is a simple and reliable quantification that does not require these certified references.¹⁹⁾ However, it is difficult to obtain the SR based on similar absorption maximum wavelength to the targeted analyte, stable physical properties, sufficient chromatographic separation, high-purity, inexpensive, and easily accessible for their use in LC detection.²⁰⁾ Thus, in our previous study, the SR proposition was investigated in the SR-LC assay of basic lignans based on a similar synthetic structure.²¹⁾ Synthetic SR was designed for the simultaneous estimation of sesamin, sesamol, episesa-

* To whom correspondence should be addressed. e-mail: kinoue@fc.ritsumei.ac.jp

Fig. 1. Structures of Curs (a) and Approach of MR Design by Chemical Methylation (b)

min, and sesamol based on the 1,3-benzodioxole modified with alkyl groups from inexpensive sesamol material.²²⁾ Our chemical SR design would be interested in an effective strategy for SR-LC assay for the simultaneous estimation of analogs to natural compounds.

It has been reported that Cur 1, Cur 2 and Cur 3 could be separated and analyzed by conventional LC assay with these certified references.^{22–25)} However, the SR-LC assay is not applied without expensive or purified references. Curs are naturally occurring yellow-orange pigments in physical conditions.²⁶⁾ Dynamics of the excited state (the *cis*- and *trans*-Diketo Forms and the Keto-Enol Tautomeric Equilibrium) of Cur 1 has been observed in a wide variety of solvents using absorption spectroscopic techniques.²⁶⁾ If the development of SR-LC assay for these polyphenolic compounds such as Curs, there are many difficulties to select and/or find useful references based on the performance of detection wavelength, retention time and physical stability. Moreover, Cur 1 standard is easily accessible through common reagent compared with Cur 2 and 3. Thus, it would be needed to propose with versatile procedure for polyphenolic compounds such as Curs. In the first and unique study, the synthesis of methylated reference (MR) of a polyphenolic compound is applied for SR-LC assay. Chemical methylation of the polyphenolic compound is a simple reaction, low-cost way, and attenuated of tangled process. In addition, the synthesis of MR would be shown to improve detection wavelength, stirred retention time and physical stability. In our idea, it can be used with methylated Cur 1 that Curs in turmeric products are simultaneously estimated without respective calibration curves. Already, the SR-LC assay based on high-speed countercurrent chromatography (HSCCC) purification was employed for the estimation of xanthomonasin A and B in *Monascus* yellow colorant.²⁰⁾ HSCCC

Fig. 2. Typical LC and HSCCC Chromatograms of Turmeric Powder and MR at 405 nm

(a) LC chromatogram of turmeric powder. (b) HSCCC chromatogram of turmeric powder. (c) LC chromatogram of MR. In LC condition, the TSK-GEL ODS 100 V column (4.6 × 150 mm, 5.0 μm, Tosoh Co.) was used. The mobile phase consisted of 0.1% FA in water/acetonitrile. The flow rate was 1.0 mL/min. In HSCCC condition, the retention of the stationary phase (50%), the *n*-hexane/chloroform/methanol/water (5/10/7.5/2.5, v/v/v/v) two-phase solvent system and the flow rate (1.5 mL/min) were configured.

provided a useful and effective purification system for natural ingredients from complex materials. In our previous experiment, HSCCC can be applied for the effective purification of Cur 1, Cur 2, and Cur 3 from turmeric materials.¹¹⁾ Thus, two ideas with MR and HSCCC were merged to apply a novel quantity of Curs using SR-LC assay for the quality assessment of turmeric products. Our approach is constructed with the synthesis of MR to develop an SR-LC assay for a wide variety of polyphenolic compounds without these certified references.

Experimental

Chemicals and Reagents The turmeric powder was obtained from San-Ei Gen F.F.I Co., Inc. (Osaka, Japan). Curcumin standard, methanol, acetonitrile, formic acid (FA, HPLC grade, 99%), and 1,4-BTMSB-*d*₄ were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Deuterated acetone (acetone-*d*₆) was purchased from Merck KGaA (Darmstadt, Germany). Acetone and potassium carbonate were obtained from Nacalai Tesque (Osaka, Japan). Purified water was obtained using a PURELAB flex 5 system from ELGA Co., Inc. (London, U.K.). Stock solutions were adjusted using methanol.

Concentrated solutions of Curs were diluted as required by adding acetonitrile/water (50/50, v/v) for LC assay.

LC Instrument and Condition The LC system comprised an LC-20AD pump, SIL-20AC autosampler, CBM-20A controller, SPD-M20A and RF-10AXL detectors, and a CTO-10AS column oven (Shimadzu Co., Kyoto, Japan). A TSK-GEL ODS-100 V column (4.6 × 150 mm, 5.0 μm; Tosoh Co., Tokyo, Japan) was used for the separation of analytes. The mobile phase in LC analysis was composed of 0.1% FA in water (solvent A)/0.1% FA in acetonitrile (solvent B). The iso-

Fig. 3. The SR-LC Chromatograms with PDA (405 nm) of Curs and MR in Curcumin GS (a)–(c), Curcumin W (d)–(f) and Turmeric Pigment Product (g)–(i)

MR levels were 25 $\mu\text{mol/L}$ (a,d, and g), 50 $\mu\text{mol/L}$ (b, e, and h) and 100 $\mu\text{mol/L}$ (c, f, and i).

cratic condition was as follows: 50% solvent B at 20 min. The system was operated at a flow rate of 1.0 mL/min. The column temperature was set to 40°C, and the volume of the injected sample was 10 μL . The analytes were monitored by UV/visible absorbance in the range of 200 to 500 nm (monitoring wavelength: 405 nm). In addition, for the instrumental validation, we used other LC instruments such as Waters H-Class (Waters, MA, U.S.A.) and Hitachi High-Tech Science Chromaster (Hitachi Ltd., Tokyo, Japan) systems.

Synthesis of MR for Curs Curcumin (0.5 g, 1.4 mmol) and methyl iodide (4.0 g, 10 mmol) were transferred to the flask. An additional 15 mL of acetone and potassium carbonate (0.5 g, 3.6 mmol) and refluxed at 80°C for 24 h and purified using a single-channel automated flash chromatography system (Smart Flash EPCLC AI-580S, Yamazen Corporation, Osaka, Japan). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 7.69 (2H, d, $J = 15.5$ Hz), 7.13 (2H, dd, $J = 8.4, 2.0$ Hz), 7.01 (2H, d, $J = 2.0$ Hz), 6.84 (2H, d, $J = 8.4$ Hz), 6.65 (2H, d, $J = 15.5$ Hz), 3.90 (12H, s), 1.48 (6H, s). For the evaluation of purity, the LC chromatogram of MR was obtained with the above-described condition. MS spectra were recorded on a Waters Xevo TQD triple quadrupole mass spectrometer with an electrospray ionization (ESI) source in the positive mode. The ionization source conditions were as follows: capillary voltage of 2.0 kV, extractor voltage of 3 V, RF lens voltage of 2.5 V, source temperature of 150°C, and desolvation temperature of 400°C. The cone and desolvation gas flows were 50 and 800 L/h, respectively, and were obtained using a nitrogen source (N_2 Supplier Model 24S, Anest Iwata Corporation, Yokohama, Japan). MS and daughter ion scan ranges were adjusted over the range m/z 100 to 600. Cone voltage was set to 20 V. The detectable ion is $[\text{M} + \text{H}]^+$ m/z 425.

HSCCC Instrument and Condition HSCCC was performed using an Easy-Prep CCC (multi-layer coil planet centrifuge, Kutsuwa Co., Ltd., Hiroshima, Japan) with a 7.6 cm orbital radius that produces a synchronous type-J planetary

Table 1. RMS Values of Curs

Analytes	Range ($\mu\text{mol/mL}$)	RMS values based on 3 d
Cur 1	0–100	9.0 ± 0.06
	0–20	8.8 ± 0.12
	20–100	9.0 ± 0.06
	Mean (RSD, %)	8.9 (1.38%)
Cur 2	0–100	9.7 ± 0.06
	0–20	9.6 ± 0.12
	20–100	9.7 ± 0.06
	Mean (RSD, %)	9.6 (0.90%)
Cur 3	0–100	9.1 ± 0.15
	0–20	8.8 ± 0.23
	20–100	9.1 ± 0.15
	Mean (RSD, %)	9.0 (2.4%)

motion with a maximum speed of 1500 rpm. This centrifuge was equipped with three column holders and multilayer coiled columns. Each multilayer coiled column on the holder consists of nine coiled layers of 1.6 mm i.d. polytetrafluoroethylene tubing with a capacity of about 120 mL. All three columns are connected in series to provide a total capacity of about 350 mL. The fractionation system (PU 714M LC pump, UV702 detector, SC 762 system controller and PLC 761 fraction collector) was obtained from GL Science Inc. (Tokyo, Japan). The two-phase system, composed of *n*-hexane/chloroform/methanol/water (5/10/7.5/2.5, v/v/v/v) at room temperature, was thoroughly equilibrated.¹¹⁾ The phases were separated before use. First, the coiled column was filled with the upper stationary phase. The separation was performed out in a reversed phase, with the column initially filled with a less polar upper stationary phase. Second, 20 mL of methanol was added to 200 mg of turmeric powder, and then mixed. After

Table 2. Application of Turmeric Products Using RMS and Absolute Calibration Methods

Quantitation approach	RMS concentration ($\mu\text{mol/L}$)	Curcumin GS (Mean $\pm$ S.D., $\mu\text{mol/L}$)			Curcumin W (Mean $\pm$ S.D., mmol/L)			Turmeric pigment product (Mean $\pm$ S.D., mmol/L)		
		Cur 1	Cur 2	Cur 3	Cur 1	Cur 2	Cur 3	Cur 1	Cur 2	Cur 3
RMS method	25	1.2 ± 0.7	0.5 ± 0.7	0.5 ± 0.7	1.2 ± 0.7	0.5 ± 0.7	0.5 ± 0.7	1.2 ± 0.7	0.5 ± 0.7	0.5 ± 0.7
	50	1.2 ± 0.3	0.5 ± 0.4	0.5 ± 0.2	1.2 ± 0.3	0.5 ± 0.4	0.5 ± 0.2	1.2 ± 0.3	0.5 ± 0.4	0.5 ± 0.2
	100	1.3 ± 1.2	0.6 ± 1.0	0.6 ± 0.2	1.3 ± 1.2	0.6 ± 1.0	0.6 ± 0.2	1.3 ± 1.2	0.6 ± 1.0	0.6 ± 0.2
Absolute calibration method		1.3 ± 0.1	0.5 ± 0.4	0.5 ± 0.6	1.3 ± 0.1	0.5 ± 0.4	0.5 ± 0.6	1.3 ± 0.1	0.5 ± 0.4	0.5 ± 0.6

S.D., standard deviation.

centrifuging at 10000rpm for 10min, the supernatant was evaporated to dryness, and then the residue was dissolved in 2.0mL of each phase. Finally, turmeric powder solution was loaded onto the column. The column was rotated at 1000rpm while the lower more polar mobile phase was pumped into the head of the column at a flow rate of 1.5mL/min. Then, the fractions (Cur 1, Cur 2 and Cur 3) were recovered, evaporated to dryness, and assayed using the LC assay described above, monitored at 405nm to assess the purity of the fractions.

qNMR Instrument and Condition The NMR instrument is 600MHz spectrometer (JNM-ECA; JEOL Ltd., Tokyo, Japan). The qNMR reference solution was prepared by dissolving 2.0mg of 1,4-BTMSB- d_4 in 2.0mL of acetone- d_6 . Calibration of the 1,4-BTMSB- d_4 concentration in the qNMR reference solution was performed as follows. 10mg of each Curs and MR were dissolved in 1.0mL of acetone- d_6 . The 0.6mL of the resulting solution was transferred to an NMR test tube. The qNMR conditions were as follows: -5 to 15ppm spectral width, 65536 data points, 90° flip angle, 60s pulse delay, 8–16 scans, probe at room temperature, and no sample spin. The obtained ^1H -NMR data was entered into the selected analysis software (Purity pro qNMR ANALYSIS software, JEOL, Tokyo, Japan).

Calculation of RMS Samples removed from the NMR tube were diluted with acetonitrile /water (50/50, v/v), and analyzed by LC assay. Absolute calibration curves were prepared at various concentrations width of Curs and MR (0 to $100\mu\text{mol/L}$). The peaks areas when the concentrations of analytes were $0\mu\text{M}$, were set to point of origin. LC analysis was performed three times for each sample, and the average was obtained. RMS values were the ratio of the slope of the absolute calibration curve of each Curs. The following equation was used to determine the RMS values:

$$\text{RMS value of Curs} = \text{Slope of each Curs} / \text{Slope of MR}.$$

Quantity of Curs in Turmeric Products by SR-LC Assay

The Curs were quantified under the above-described LC conditions based on absolute calibration and RMS methods. For absolute calibration method, these concentrations of Curs in samples were calculated using the above calibration curve. For RMS method, these concentrations of Curs in samples were calculated using RMS values. For application samples, we selected turmeric products such as Curcumin GS (Daiwa Dye-stuff Mfg. Co., Ltd., Saitama, Japan), Curcumin W (Hodogaya Chemical Co., Ltd., Tokyo, Japan), and Turmeric pigment product (San-Ei Gen F.F.I., Inc., Osaka, Japan). These samples were diluted with acetonitrile/water (50/50, v/v), and added MR (25, 50, and $100\mu\text{mol/L}$). In addition, the application of supplements was used of solid types (5 kinds). These samples were extracted with methanol and centrifuged at 10000rpm for

Fig. 4. The Concentration Levels ($\mu\text{mol/L}$) of Cur 1 (a), Cur 2 (b) and Cur 3 (c) in Five Supplemental and Seasoning Products Using Three Different Instruments Such as Shimadzu (White), Waters (Gray), and Hitachi, Co. (Black)

10min. The supernatant layers were diluted with acetonitrile/water (50/50, v/v), and added MR ($50\mu\text{mol/L}$).

Results and Discussion

LC Analysis and HSCCC Purification of Curs from Turmeric Powder A previous study developed the LC analysis

and HSCCC purification of Curs from turmeric powder.¹¹⁾ The LC chromatogram of turmeric powder was shown in Fig. 2-(a) using this previous condition and is very efficient and adequate for the LC assay of Curs. Furthermore, the HSCCC chromatogram of turmeric powder is shown in Fig. 2-(b) and is useful for the purification of Curs (Cur 1, Cur 2 and Cur 3). These purified Curs were confirmed using negative MS (Cur 1; $[M-H]^-$ m/z 367, Cur 2; $[M-H]^-$ m/z 337, Cur 3; $[M-H]^-$ m/z 307) and LC assay (405 nm; purity >99%) (Supplementary Fig. S1).

The Approach of MR Design for SR-LC Assay of Curs
For the development of SR-LC assay, characteristic reference should be needed to investigate the simultaneous separation of Curs based on the above LC condition. We previously described several references based on the same wavelength and good separation (different retention times).^{20,21)} Indeed, for catechins, the flavonoid structure strongly interacted with the C_{18} polymers of the column; thus, altering specific functional groups was challenging at a similar retention time.¹⁶⁾

Fig. 5. The SR-LC Chromatograms with PDA (405 nm) of Curs and MR in Five Supplemental and Seasoning Products Using Shimadzu (Left Side), Waters (Center Side), and Hitachi (Right Side)

(a, f, k) Supplemental product 1. (b, g, l) Supplemental product 2. (c, h, m) Supplemental product 3. (d, i, n) Seasoning product 4. (e, j, o) Seasoning product 5.

We speculated that at the same wavelength, a change in the polarity-sensitive retention is observed, leading to good separation and suitable retention time. Thus, a novel approach is attempted to make the MR for retaining the key structure of Curs. Chemical methylation of the polyphenolic compound is a very easy, simple, and inexpensive procedure. In this study, Cur 1 was reacted with CH_3I in an alkaline solvent and reaction efficiency of 5.92% (Fig. 1-(b)). The MR exhibited a moderate retention time (about 17 min) under the described LC conditions (Fig. 2-(c)). The chemical methylation of phenolic analytes will be a useful, inexpensive, and diverse idea for the development of SR-LC assay of various natural products. Following the alternative SR, the purities of these compounds were evaluated using a qNMR method. The results showed several compounds such as MR (80.4%), Cur 1 (74.2%), Cur 2 (64.8%), and Cur 3 (71.7%), respectively. The reproducible values of all standards were relative standard deviation (RSD) $< 1.0\%$ ($n = 3$). Based on these solvents from qNMR, the approximate amounts of the MR and Curs in the NMR tube (0.8 mL) were calculated and adjusted to the calibration curves (Supplementary Fig. S2, $r^2 > 0.998$). Using the adjusted solutions of Curs and MR, the averaged RMS values (Cur 1 = 8.92, Cur 2 = 8.97 and Cur 3 = 9.61 for 3 d) were calculated based on the calibration slopes of six ranges, *i.e.*, 0 (origin) to 20 and 100 $\mu\text{mol/L}$ using the optimal LC conditions at 405 nm (Table 1). To examine the accuracy and applicability of the SR-LC assay, the concentrations of Curs in three turmeric products for food additives were determined by three different levels of MR. The SR-LC chromatograms of turmeric are illustrated in Fig. 3. Table 2 shows the definite quantities of Curs obtained using LC assay, calculated by absolute calibration as well as by three levels (25, 50 and 100 $\mu\text{mol/L}$) of MR calibrations. The established differential definite quantities of Curs in three turmeric products for food additives were similar using RMS and absolute calibration methods based on $\text{RSD} < 5.10\%$. In addition, based on the RMS of MR, various concentration levels of Curs are simply derived using the peak ratio by a single LC running.

Application and Validation of SR-LC Assay for the Quantity of Curs in Supplements For the evaluation of SR-LC methodology, these five supplemental and seasoning products from Japanese markets were analyzed using three different instruments (Shimadzu, Waters, and Hitachi). Masumoto *et al.* reported that analyte concentrations in real products were determined using three instruments to test the accuracy and applicability of the HPLC condition.¹⁵⁾ They were presuming that the quantitative value is directly affected by the ability of individual specificity. Thus, we investigated the detectable concentration levels of Curs in five supplemental and seasoning products (Nos. 1–5) using three instruments (Fig. 4). In addition, these chromatograms of Curs and MR were shown in Fig. 5. These results showed that variabilities of Cur 1, Cur 2 and Cur 3 were $\text{RSD} < 6.1\%$, $< 5.7\%$, and $< 4.3\%$ based on three instruments, respectively. Thus, these results support the validity of the developed SR-LC assay with RMS for the quantitative analysis of natural ingredients such as Curs from complex materials.

Conclusion

The useful and wide versatility of MR to improve applications in the SR-LC assay based on RMS. It was indicated with

MR that Curs in various turmeric products were determined without respective calibration curves using authentic standards. In addition, for the RMS to be demonstrated, HSCCC purification is necessary to separate Curs. An SR quantitative method based on HSCCC purification, qNMR evaluation, and RMS conversion was first developed for the determination of Curs in various food products. In addition, this novel quantification based on the easy-to-use SR-LC assay with RMS could be further used to analyze the main components and/or impurities substances in natural materials for safety and quality assessment.

Acknowledgments This study was supported by a Grant from the Japan Food Chemical Research Foundation and a Health Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare, Japan.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

References

- 1) Memarzia A., Khazdair M. R., Behrouz S., Gholamnezhad Z., Jafarnejad M., Saadat S., Boskabady M. H., *Biofactors*, **47**, 311–350 (2021).
- 2) Heidari Z., Mohammadi M., Sahebkar A., *Adv. Exp. Med. Biol.*, **1308**, 127–136 (2021).
- 3) Singh R., Shaik L., Mehra I., Kashyap R., Surani S., *Med. J.*, **14**, 79–86 (2020).
- 4) Pluskota-Karwatka D., Hoffmann M., Barciszewski J., *J. Pharm. Anal.*, **11**, 383–397 (2021) doi: 10.1016/j.jpha.2021.03.012.
- 5) Priyadarsini K. I., *Molecules*, **19**, 112 (2014).
- 6) Akter J., Hossain M. A., Takara K., Islam M. Z., Hou D. X., *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **215**, 9–17 (2019).
- 7) Thanawala S., Shah R., Alluri K. V., Somepalli V., Vaze S., Upadhyay V., *J. Pharm. Pharmacol.*, **73**, 816–823 (2021).
- 8) Carolina Alves R., Perosa Fernandes R., Fonseca-Santos B., Damiani Victorelli F., Chorilli M., *Crit. Rev. Anal. Chem.*, **49**, 138–149 (2019).
- 9) Kukula-Koch W., Grabarska A., Luszczycki J., Czernicka L., Nowosadzka E., Gumbarewicz E., Jarzab A., Audo G., Upadhyay S., Glowinski K., Stepulak A., *Phytother. Res.*, **32**, 933–942 (2018).
- 10) Song W., Qiao X., Liang W. F., Ji S., Yang L., Wang Y., Xu Y. W., Yang Y., Guo D. A., Ye M., *J. Sep. Sci.*, **38**, 3450–3453 (2015).
- 11) Inoue K., Nomura C., Ito S., Nagatsu A., Hino T., Oka H., *J. Agric. Food Chem.*, **56**, 9328–9336 (2008).
- 12) Pan Y., Ju R., Cao X., Pei H., Zheng T., Wang W., *J. Sep. Sci.*, **43**, 1586–1592 (2020).
- 13) Tayyem R. F., Heath D. D., Al-Delaimy W. K., Rock C. L., *Nutr. Cancer*, **55**, 126–131 (2006).
- 14) Masada S., Hosoe J., Arai R., Demizu Y., Hakamatsuka T., Goda Y., Uchiyama N., *Chem. Pharm. Bull.*, **69**, 573–580 (2021).
- 15) Masumoto N., Ishizuki K., Nishizaki Y., Ohtsuki T., Kuroe M., Yamazaki T., Numata M., Matsufuji H., Sugimoto N., Sato K., *Chem. Pharm. Bull.*, **69**, 18–25 (2021).
- 16) Takahashi M., Nishizaki Y., Masumoto N., Sugimoto N., Sato K., Inoue K., *J. Sci. Food Agric.*, **101**, 3804–3810 (2020) doi: 10.1002/jsfa.11013.
- 17) Ohtsuki T., Matsuoka K., Fuji Y., Nishizaki Y., Masumoto N., Sugimoto N., Sato K., Matsufuji H., *PLOS ONE*, **15**, e0243175 (2020).
- 18) Kitamaki Y., Saito N., Yamazaki T., Otsuka S., Nakamura S., Nishizaki Y., Sugimoto N., Numata M., Ihara T., *Anal. Chem.*, **89**,

- 6963–6968 (2017).
- 19) Nishizaki Y., Sato-Masumoto N., Yokota A., Mikawa T., Nakashima K., Yamazaki T., Kuroe M., Numata M., Ihara T., Ito Y., Sugimoto N., Sato K., *Food Addit. Contam. Part A*, **35**, 838–847 (2018).
- 20) Takahashi M., Nishizaki Y., Sugimoto N., Sato K., Inoue K., *J. Chromatogr. A*, **1555**, 45–52 (2018).
- 21) Takahashi M., Nishizaki Y., Morimoto K., Sugimoto N., Sato K., Inoue K., *Sep. Sci. Plus*, **1**, 498–505 (2018).
- 22) Wichitnithad W., Jongaroonngamsang N., Pummangura S., Rojsitthisak P., *Phytochem. Anal.*, **20**, 314–319 (2009).
- 23) Syed H. K., Liew K. B., Loh G. O., Peh K. K., *Food Chem.*, **170**, 321–326 (2015).
- 24) Chao I. C., Wang C. M., Li S. P., Lin L. G., Ye W. C., Zhang Q. W., *Molecules*, **23**, 1568 (2018).
- 25) Mudge E. M., Brown P. N., *J. AOAC Int.*, **101**, 203–207 (2018).
- 26) Mukerjee A., Sørensen T. J., Ranjan A. P., Raut S., Gryczynski I., Vishwanatha J. K., Gryczynski Z., *J. Phys. Chem. B*, **114**, 12679–12684 (2010).