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A sensitive LC-MS assay using derivatization with boron trifluoride to quantify curcuminoids in biological samples

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ab.2020.113636.

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Abstract

A procedure is described to measure curcumin (C), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), tetrahydrocurcumim (TC) and their glucuronidated metabolites (CG, DMCG, and BDMCG) in plasma, brain, liver and tumor samples. The procedure involves converting the analytes to their boron difluoride derivatives and analyzing them by combined liquid chromatography coupled to an ion trap mass spectrometer operating in the negative ion MS^n scan mode. The method has superb limits of detection of 0.01 nM for all curcuminoids and 0.5 nM for TC and the glucuroniated metabolites, and several representative chromatograms of biological samples containing these analytes are provided. In addition, the pharmacokinetic profile of these compounds in one human who daily consumed an over-the-counter curcuminoid product shows the peak and changes in circulating concentrations achieved by this mode of administration.

Keywords

Curcumin; Curcuminoids; Liquid chromatography coupled mass; spectrometry (LCMS); Pharmacokinetics; Boron trifluoride (BF_3)

1. Introduction

Turmeric, a spice derived from the rhizome of *Curcuma longa* L., has been used for centuries as traditional medicine to treat a variety of human ailments and physical wounds in China and India [1]. The major active component of turmeric is thought to be the yellow pleiotropic polyphenol curcumin (C). C is reported to possess a wide range of bioactivities, such as anti-inflammatory, anti-oxidant, anti-proliferative, and anti-angiogenic effects [2–4]. Demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) are closely related bioactive polyphenols also found in turmeric, albeit in lower abundance. They are structurally similar to C but lack one or both methoxy groups, respectively. Findings from a number of studies have demonstrated bioactivities for both DMC and BDMC, and in some cases their potency is equivalent or even greater than that of C [4–7]. Also, three independent studies found that DMC and BDMC can synergistically enhance the effect of C, although the mechanism is unknown [8–10]. Collectively referred to as curcuminoids, C, DMC, and BDMC are variously abundant in turmeric-based dietary supplements as well as in commercially available partially purified curcuminoid mixtures frequently used for biomedical research.

There is a growing interest in the therapeutic and disease-preventing potentials of C and related compounds. Over 166 privately or publicly funded clinical trials have been conducted or are on-going as of October 2019 [11]. However, there is also skepticism about the actual *in vivo* benefits of curcuminoids associated with their low bioavailability following oral consumption, and rapid metabolism. Although several formulations have been developed to improve absorption and stability, curcuminoids are still subjected to rapid glucuronide and sulfate conjugation and clearance after crossing the submucosal barriers. In

order to assess pharmacological efficacy of these compounds, accurate measurement of free and conjugated C in complex biological samples, such as plasma and tissue, is critical.

The literature is replete with reports of methods for quantitating C and related compounds in plasma and other biological samples. These methods include HPLC with UV detection [12–15] and combined liquid chromatography (LC) with tandem mass spectrometry (MS/MS) in the multiple reaction monitoring mode (MRM) [16–19]. However, none of these methods cover the spectrum of major curcuminoids and metabolites that is needed for a comprehensive assay, and from our experience none have the sensitivity necessary to measure low (down to 50 fmol/sample) amounts of these compounds in small biological samples available from animal or human studies.

We addressed this issue by converting C, DMC, and BDMC to their boron difluoride (BF_2) complexes. The conversion reaction rapidly goes to completion at room temperature (Fig. 1), and the detection sensitivity of the BF_2-curcuminoid complexes in negative ion electrospray ionization mass spectrometry (ESI-MS) is significantly better than that of the native molecules. The reaction has been optimized into a simple single-step process, and it is used to develop a sensitive LC-MS assay to measure free curcuminoids and their major metabolites in plasma and soft tissues. The assay has been validated, and subsequently applied to investigate the bioavailability and pharmacokinetics of curcuminoids in animal and human subjects.

2. Materials and methods

Chemicals:

Analytical grade reagents were used throughout. Suppliers of specific chemicals are indicated in the text.

Biological samples:

Human plasma.—From a healthy volunteer who orally consumed an over-the-counter curcuminoid formulation (Longvida®, Verdure Sciences, Noblesville, IN), a qualified phlebotomist collected blood into plasma collection tubes containing K_2EDTA (BD vacutainer). The tubes were centrifuged (1000 g, 15 min), and plasma was collected and stored at −80 °C.

Murine soft tissues.—Mouse liver, brain, and tumor samples were collected from animals that had been administered a proprietary turmeric formulation (PHRAD 129, Aveta Biomics, Bedford, MA). After dissection, the tissues were weighed, frozen and stored at −80 °C. Details of these experiments will be presented elsewhere.

Preparation of standard solutions:

Stock solutions of C (Sigma, St. Louis, MO), DMC (ChromaDex, Irvine, CA), BDMC (ChromaDex, Irvine, CA), and TC (Toronto Research Chemicals, Toronto, Canada) were prepared in methanol (100 nmol/mL). Curcumin β-D-glucuronide (CG, Toronto Research Chemicals, Toronto, Canada) stock solution at the same concentration was prepared in

methanol/H_2O (70/30). Working curcuminoid mixture solutions were prepared from the stock solutions by serial dilution in methanol. In addition, $^2H_6$-curcumin (D6C, Toronto Research Chemicals, Toronto, Canada) and $^2H_6$-tetrahydrocurcumin (D6TC, Toronto Research Chemicals, Toronto, Canada) solutions (internal standards, ISs) were prepared in methanol at concentrations of 0.1 and 0.5 nmol/mL, respectively. All standard solutions were stored at −20 °C in the dark.

Quality Controls:

Quality control (QC) samples at three different concentrations (QC-low, QC-middle, and QC-high) were prepared by spiking appropriate volumes of the working curcuminoid standard mixture solution to 100 μL of drug-naive human plasma. The final concentrations of C, DMC, and BDMC in QC-low, QC-medium, and QC-high samples were 0.5, 2.5, and 25 nM, respectively, while for TC and CG, the final concentrations were 5, 25, and 100 nM, respectively.

Extraction of human plasma samples:

Plasma samples were prepared in duplicate by transferring aliquots of plasma (100 μL) to 1.5 mL microcentrifuge tubes. The ISs (1 pmol D6C and 5 pmol D6TC in 10 μL of methanol) were added to each sample. The samples were briefly mixed, then treated with cold (4°C) acetic acid in methanol (10 mM, 1 mL). After rigorous mixing, incubation (4 °C, 1 h), and centrifugation (16,000 g, room temperature (RT), 5 min) supernatants were transferred to microcentrifuge tubes and dried in a vacuum centrifuge. The dried samples were tightly capped and stored at −80 °C.

Extraction of mouse liver, brain and tumor tissue samples:

Acetic acid (120 μL, 10 mM), ethyl acetate/methanol (95/5, 500 μL), and a solution of ISs (1 pmol D6C and 5 pmol D6TC in 10 μL of methanol) were added to pre-weighed (30–50 mg) frozen tissue samples in 2 mL reinforced microcentrifuge tubes (Fisher, part # 15–340-162) containing ceramic beads. The samples were homogenized in a bead mill (thrice for 10 s each time at RT). The homogenate was vigorously mixed (5 min, RT), centrifuged (16,000 g, 5 min at RT), and the supernatant was transferred to microcentrifuge tubes and dried in a vacuum centrifuge. The dried samples were tightly capped and stored at −80 °C.

BF_2-curcuminoids complex formation:

Dried samples were resuspended in glacial acetic acid (20 μL) followed by sonication (15 s) in a water bath sonicator. BF_3 reagent (20 μL, 20% boron trifluoride etherate (BF_3–OEt_2, Acros Organics, New Jersey) prepared immediately before use in glacial acetic acid) was added to the samples, which were incubated (30 min, RT) in the dark. The samples were then centrifuged (16,000 g, 5min, RT), and the supernatants were transferred to polypropylene HPLC injector vials for LC-MS analysis.

Calibration curve standards:

Simultaneously with each batch of biological samples, calibration curve standards were prepared in triplicate at increasing concentrations (0, 0.05, 0.1, 0.5, and 2.5 pmol for C,

DMC, and BDMC; 0, 0.5, 2.5, 5.0, and 10.0 pmol for CG and TC) by spiking appropriate volumes of the working curcuminoid mixture solutions to curcumin-naive human plasma (100 μL). After addition of the ISs (1 pmol D6C and 10 pmol D6TC in 10 μL methanol), the standard samples were processed together with the biological samples as described above.

LC-MS analysis of BF_2-curcuminoid complexes:

Aliquots (typically 15 μL) of the derivatized samples were injected onto a reversed phase HPLC column (Phenomenex Kinetex®, 1.7 μm, XB-C18, 100 Å, 100 × 2.1 mm, or equivalent) equilibrated in eluant A (water/formic acid, 100/0.01, v/v) and eluted (100 μl/min) with an increasing concentration of eluant B (acetonitrile/isopropanol, 50/50, v/v: min/% B, 0/15, 1.5/15, 6.5/100, 7.5/100, 8/15, and 10/15). The effluent from the column was passed directly to an electrospray ion source connected to a linear ion-trap mass spectrometer (Thermo LTQ XL) operating in the negative ion MS^n mode, in which parent ions at *m/z* 415.1 (C), 355.1 (BDMC), 385.1 (DMC), 591.1 (CG), 419.1 (TC), 421.1 (D6C) and 425.1 (D6TC) were selected and fragmented with previously optimized settings (isolation width 2.0, normalized collision energy 35, and activation Q 0.25). While C, BDMC, CG, DMC, and D6C were monitored in MS^2 mode, TC and D6TC were monitored in MS^3 mode in which the major MS^2 fragment ions at *m/z* 283 and 286 (TC and D6TC, respectively) were fragmented again for improved specificity. Data was extracted from each file for the corresponding MS^2 or MS^3 fragment ions at *m/z* 400 (C), 289 (BDMC), 415 (CG), 147 (TC and D6TC), 370 (DMC), and 403 (D6C) using a 1 Da window. Standard curves for each compound were plotted as peak area ratio (analyte peak area/IS peak area; ordinate) against concentration of each analyte in the calibration curve standards (abscissa). D6C was used as the IS for all compounds except TC, for which D6TC was used. The concentration of each analyte in the biological samples was computed by interpolating the peak area ratio of the analyte to the corresponding standard curve.

3. Results and discussion

Published procedures were initially adopted and modified to develop an assay for measuring the concentrations of C and related compounds in human plasma and serum after oral consumption of commercially-available C formulations [16–19]. However, despite repeated attempts, the level of sensitivity was inadequate for measurement of the complete pharmacokinetic profile of circulating levels of curcuminoids and their metabolites using LC/MS in either the positive or negative ion modes. These results were obtained in spite of optimizing the chromatographic and mass spectrometric conditions on both triple quadrupole (Agilent 6460) and linear ion trap mass spectrometers (Thermo LTQ XL).

BF_2 complex formation:

We tested completion of the derivatization reaction by comparing the MS and ^1H NMR spectra of the starting materials and the products after the BF_3–OEt_2 reaction. The MS spectra show a mass increase of 38 Da corresponding to loss of a proton and gain of a BF2 moiety, and no residual signals for C was observed by MS (Fig. 2) or by ^1H NMR (Fig. 3) after the reaction. The ^1H NMR spectra also show downfield shifting of most proton signals after derivatization, explained by the electron withdrawing nature of the Lewis acid.

Furthermore, the downfield shift of the signal at 10.087 ppm (assigned as the phenolic protons) explains the improved sensitivity of BF_2–C complex in negative ESI-MS due to the increased acidity of the phenolic proton coupled with stabilization provided by the Lewis acid complex of the resulting anion.

Complex stability:

The BF_2-curcumin complexes slowly hydrolyze in water and water/methanol mixtures with a half-life of a few hours at 4 °C. For applicability to an LC/MS assay on biological samples containing amounts of curcuminoids as low as 50 fmol/sample, and using an aqueous chromatographic mobile phase, it was necessary to use conditions that minimized hydrolysis. This breakdown was slowed but not averted by storing the samples at even lower temperatures (−80 °C) prior to analysis. The breakdown of the derivatives was averted by performing the reaction in glacial acetic acid and directly injecting the reaction mixture onto the LC column. Even after the injection of hundreds of samples there has been no noticeable deterioration of chromatographic reliability. Furthermore, by using a short 10 min chromatographic gradient the hydrolysis of the complexes during chromatography was minimized. The stability of BF_2-curcuminoid complex was tested to validate the assay for overnight analysis. Eight QC-mid samples were prepared, stored tightly capped to minimize contact with atmospheric moisture in the autosampler at 20 °C, and each sample was analyzed every hour for up to 7 h. Except for BF_2-TC, the curcuminoid complexes were stable throughout the 7 h test period with no significant decline in peak intensity (Supplementary Fig. 1). In the case of BF_2-TC, there was a slight loss of signal intensity during the 7-h, but this would be corrected by use of the heavy isotope labeled internal standard (D6TC).

BF_2-curcuminoid mass spectral characteristics:

The negative ion ESI mass spectra of the BF_2 complexes of C, D6C, BDMC, DMC, TC, D6TC, and CG all showed intense ions corresponding to the $[M - H]^-$ parents (Fig. 1, Table 1). Following collision-induced dissociation (CID), each BF_2-curcuminoid produced unique product ions suitable for use in quantitative analyses (Table 1). Mass losses caused by fragmentation were 15 Da (CH_3) for C and DMC, 18 Da (C^2H_3) for D6C, 66 Da (BF_2OH) for BDMC, 176 Da (glucuronic acid residue, $C_6H_8O_6$) for CG, and sequential losses of 136 Da (2-methoxy-4-methylphenol, $C_8H_8O_2$) and 139 Da ($^2H_3$-2-methoxy-4-methylphenol, $C_8H_5{}^2H_3O_2$) for TC and D6TC, respectively.

Relative response of C and BF_2–C:

Although the BF_2 complexes have already been used as a way to purify curcuminoids and related compounds [20], as we attempted to modify the phenolic groups on curcumin via Mitsunobu reaction, an observation emerged that the BF_2-curcuminoid complex is more prone to deprotonation at the phenolic group than the native counterpart. Following this observation, it also became evident that the BF_2–C complex exhibits a better response in negative ion ESI-MS. To compare the relative responses for underivatized and derivatized C, aliquots (100 μL) of a plasma extract were spiked with varying amounts of C. Some of the aliquots were taken to dryness in a vacuum centrifuge, resuspended in 50 μL of methanol, and analyzed for underivatized C using previously optimized conditions in the negative ion

mode (parent *m/z* 367 → fragment *m/z* 217). Other aliquots of the spiked plasma extracts were taken to dryness in a vacuum concentrator, derivatized with BF_3–OEt_2 and analyzed for BF_2–C as described above. The results from these matched series of samples showed an average 28-fold increase in peak area for the complex over the underivatized compounds across the range of 7.5 fmol–15 pmol injected (Fig. 4).

Assay validation: extraction recovery.

From human plasma, using the procedure described above, the recovery of the curcuminoids was determined by comparing peak intensities from the QC samples (QC-low, mid, and high) to peak intensities from curcumin-naïve control human plasma spiked post-extraction with corresponding amounts of standards. Both QC and post-extraction spiked samples were analyzed in triplicate at each concentration level. The extraction recovery from plasma was greater than 80% for all compounds, including the glucuronide, at all tested concentrations (Table 2, upper panel).

The extraction recovery of the curcuminoids from brain tissue was determined by comparing peak intensities from pre- and post-extraction spiked curcumin-naïve mouse cerebellum tissue. The results show excellent recoveries (> 80%) for all compounds except for the glucuronide conjugate (Table 2, lower panel). The poor recovery of CG is presumably attributable to the more hydrophilic nature of the compound resulting in poor partitioning into the ethyl acetate phase. Homogenizing tissue in different solvents in some cases improved the CG recovery, but resulted in loss of recovery for other compounds. For example, curcumin recovery was about 30% when the brain tissue was homogenized in methanol. Because of greater importance assigned to quantitation of the unconjugated curcuminoids, we decided to continue with using ethyl acetate to extract soft tissues.

Assay validation: accuracy and precision.

These parameters were determined by analyzing nine independent QC samples (three each QC-low, QC-mid, and QC-high) along with calibration curve standards in the same batch using D6C as the sole internal standard. The measured concentrations of individual analytes in the QC samples were compared to the nominal concentration in corresponding replicates. Accuracy for all compounds at all concentrations tested was close to 100% except for TC, again indicating the need for dedicated internal standards for precise quantitation (Table 3).

Assay validation: carry over.

This was determined by measuring peak areas of each analyte after solvent (glacial acetic acid) injections (15 μL) following multiple injections of QC-high samples and the most concentrated calibration curve standard. No detectable carry-over was observed. However, blank solvent injections are routinely included after every 10 sample injections to safeguard against the unlikely event of significant carry over.

Assay validation: limit of detection (LoD), limit of quantitation (LoQ), and practical LoQ.

The general convention for estimating LoD or LoQ is by calculating the mean background signal from blank control samples plus 3 or 10 times the standard deviation of the mean, respectively. Using these formulae, the calculated LoD and LoQ of C, DMC, and BDMC

were 0.01 nM and 0.05 nM, respectively. For TC and CG, the calculated LoD and LoQ were 0.5 nM and 1 nM, respectively. However, we used five to ten times the calculated LoQ as a practical LoQ (0.5 nM for C, DMC, and BDMC; and 5 nM for CG and TC) to ensure maximal reliability and accuracy of quantitation.

Assay validation: freeze-thaw cycle stability.

Because samples (plasma and tissue) are frequently stored frozen at −80 °C until analyzed, it was important to determine the stability of the native compounds during multiple freeze thaw cycles. One pmol of each analyte was added to 100 μL of phosphate buffered saline (PBS) and human plasma, and the samples were subjected to multiple freeze (−80 °C) thaw (RT) cycles before being processed for LC-MS analysis. The experiment was conducted without the addition of IS and the results were based on absolute signal intensities. In both PBS and plasma, all curcuminoid and related compounds tested were stable after multiple freeze-thaw cycles, with no sign of degradation (Supplementary Fig. 2).

Representative chromatograms.

When injected at their practical LoQ in the milieu of human plasma, the curcuminoids (C, DMC and BDMC) and their metabolites (for which standards are available, TC and CG) all showed intense signals (Figs. 5A and 6A, upper three traces, respectively). Extracts of plasma from a volunteer who orally consumed four tablets of a commercially available over-the-counter curcuminoid formulation (Longvida®, 230 mg of curcuminoids per 1000 mg tablet) daily for 17 consecutive days, showed strong signals for all three curcuminoids (Fig. 5B) and their metabolites (Fig. 6B, top three traces). An extract of a murine tumor following oral administration of a different turmeric formulation (PHRAD 129, dose = 100 mg/kg, daily) showed strong signals for C, DMC, BDMC (Fig. 5C) and TC (Fig. 6C, upper two traces), although there was no peak for CG (Fig. 6C, third trace). Extracts from the brain (Supplementary Fig. 3B) and liver (Supplementary Fig. 3C) of mice that had been administered the same turmeric herbal supplement showed measurable peaks for C and DMC in brain and C, DMC, and BDMC in liver. There were no detectable peaks for any of the glucuronides in these samples (Supplementary Figs. 4B and C).

The loss of the glucuronic acid moiety (176 Da, Table 1) from CG during CID suggested that this could be a common feature of the fragmentation pattern of other curcuminoid glucuronides, and prompted the search for similar losses from the predicted molecular ions of the glucuronides of DMC, BDMC (Table 1) and TC. This search resulted in appearance in the same region of the chromatograms of intense peaks tentatively assigned as the glucuronides of DMC and BDMC (DMCG and BDMCG, respectively) in the resulting ion traces (Fig. 6B, lower two traces). However, peaks for these tentatively-assigned metabolites were only detected in the human plasma, and were not detected in the murine tumor (Fig. 6C lower two traces), brain or liver samples (Supplementary Fig. 4B &C, lower two traces). Using this assay, we have already reported the detection and relative quantitation of DMCG in a human tumor after oral administration of a turmeric extract, although BDMCG was not detected in this sample [21].

In vivo temporal profile of plasma curcuminoids and metabolites in a human volunteer.

The *in vivo* pharmacokinetics of an orally administered curcuminoid formulation were explored in a single volunteer who orally consumed four tablets of a commercially available over-the-counter curcuminoid formulation (Longvida®, 230 mg of curcuminoids per 1000 mg tablet) each day for 17 consecutive days. Analysis of the blood collected at 0, 1, 2, 3, 4, and 5 h after compound ingestion on Day 1 and 0, 1, 2, 3, and 4 h on Day 17 was performed. The results show unexpectedly complex pharmacokinetic profiles (Fig. 7), presumably created in part by the time dependency of gastrointestinal absorption followed by appearance in plasma. Further complications might result from multiple elimination processes. While a detailed analysis of the pharmacokinetic profile of these compounds is beyond the scope of this communication (the main objective being a description of this new method of analysis), and bearing in mind that the data is derived from a single experiment with one subject, some observations are warranted. It appears that all compounds (curcuminoids and their metabolites) were detected 1-h following drug administration on both Days 1 and 17. On Day 1, measured concentrations were up to 40 and 250 nM for the curcuminoids and metabolites, respectively; on Day 17 the concentrations were increased significantly up to 250 and 500 nM for the curcuminoids and metabolites, respectively. The metabolites were about 10-fold more concentrated than the curcuminoids on Day 1, but this difference fell to about 2-fold on Day 17. On Day 1 there is evidence of a biphasic temporal profile for both curcuminoids and the metabolites, but this reverts to a mono-phasic profile for all compounds on Day 17. There were significant circulating concentrations of the metabolites at the zero time-point on Day 17. Although these concentrations were low, they were still above the practical LoQ. The results from the Day 1 sample suggest the *in vivo* half-lives of all compounds (curcuminoids and metabolites) is significantly longer than 5 h as there was no suggestion of a decline in their circulating concentrations during the first 5 h following ingestion of the formulation. However, the time dependency of the appearance of the compounds in the plasma following entrance into the stomach and absorption into the plasma, already referred to, makes it impossible to calculate a realistic *in vivo* half-life from this experiment. Perhaps the most interesting aspect of the kinetics was the increase in bioavailability (area under the time versus concentration curves) by about 5-fold over the 17-day period. This aspect of the kinetics highlights the increase in bioavailability during the experiment, an increase that might be even greater were the regimen to continue beyond 17 days.

4. Conclusion

We have established a sensitive LC-MS assay for measuring curcuminoids and their major metabolites and demonstrated its application for analyzing various types of biological samples. The improved sensitivity of this assay enabled accurate quantitation of these compounds not only in biological fluids, but also in small amounts of precious soft tissues, and this will benefit on-going efforts to understand *in vivo* efficacy and absorption profile of curcuminoids in both animal studies and human clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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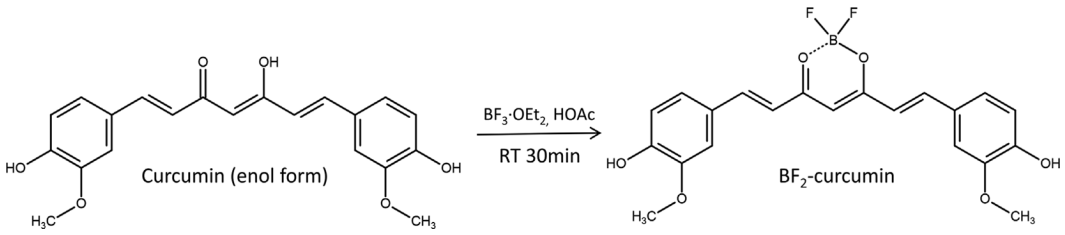


Fig. 1.

Structure of curcumin and the BF_2-curcumin product that forms after treatment with boron trifluoride etherate ($BF_3{\cdot}OEt_2$) under acidic conditions.

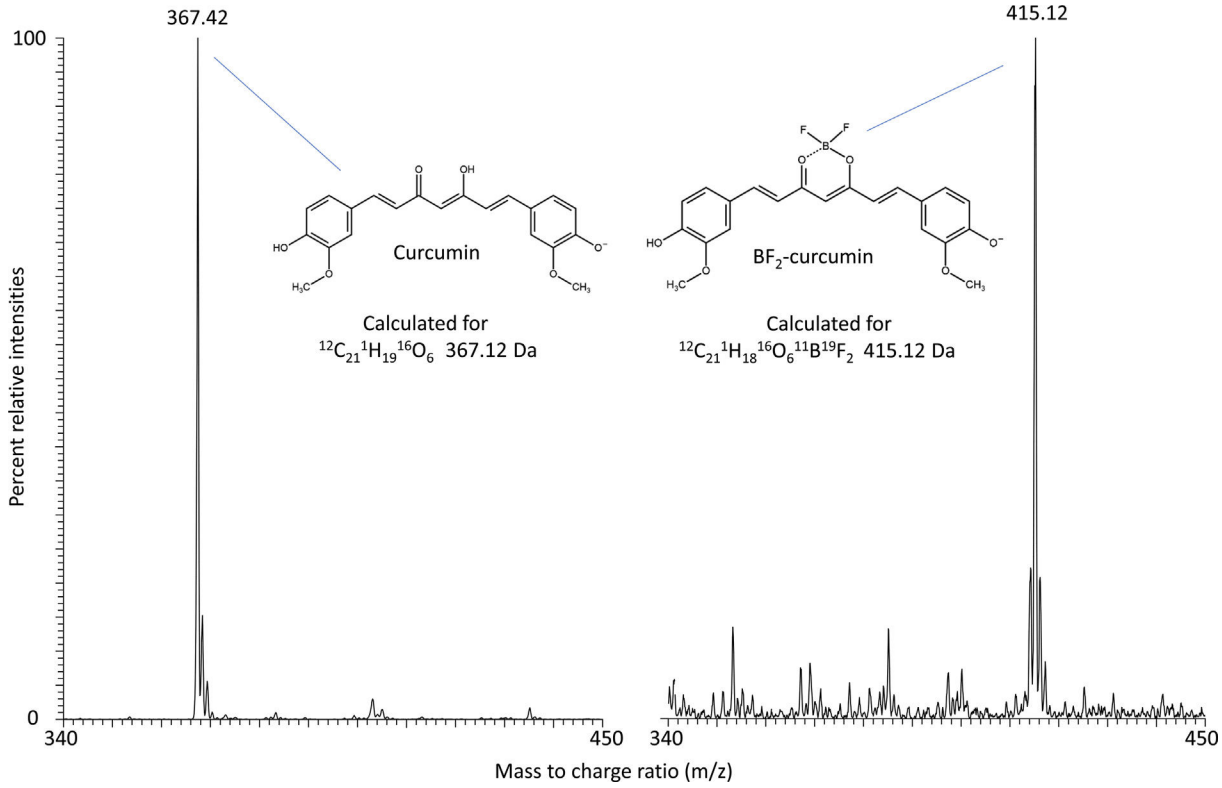
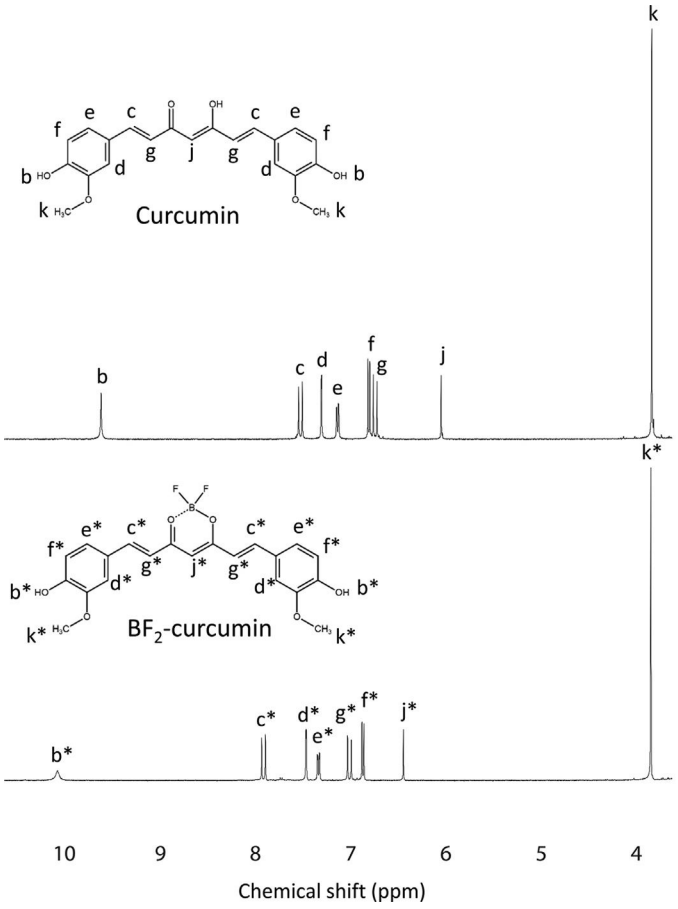


Fig. 2.

Negative ion electrospray mass spectra of curcumin (left) and BF_2-curcumin (right) collected by direct injections into an ESI source connected to a linear ion trap mass spectrometer. The concentration of the solutions used to collect these spectra were not the same with a more dilute solution used for the BF_2 derivative, and this is reflected in higher background in the spectrum.



Proton assignment	Chemical shift (ppm)	Integration
b	9.6452	2.00
c	7.562-7.522	2.28
d	7.322-7.318	1.94
e	7.162-7.137	1.92
f	6.830-6.809	2.24
g	6.772-6.7333	2.20
j	6.057	1.00
k	3.836	6.44
b*	10.087	1.75
c*	7.94-7.902	2.12
d*	7.476-7.472	2.04
e*	7.356-7.331	1.95
f*	6.888-6.867	2.12
g*	7.038-6.999	2.04
j*	6.452	1.00
k*	3.853	6.32

Fig. 3.

Expanded view of the ^{1}H NMR (400 MHz) spectra of curcumin (top) and BF_2-curcumin (bottom) showing the structural symmetry and proton splitting patterns of the two molecules. BF_2-curcumin proton assignments were made with reference to the previously described curcumin proton assignments (http://sdbs.db.aist.go.jp, using CAS 458–37-7). Samples were dissolved in $^{2}H_6$-dimethylsulfoxide (1 mg/mL) and integrations were made relative to the j and j* signals.

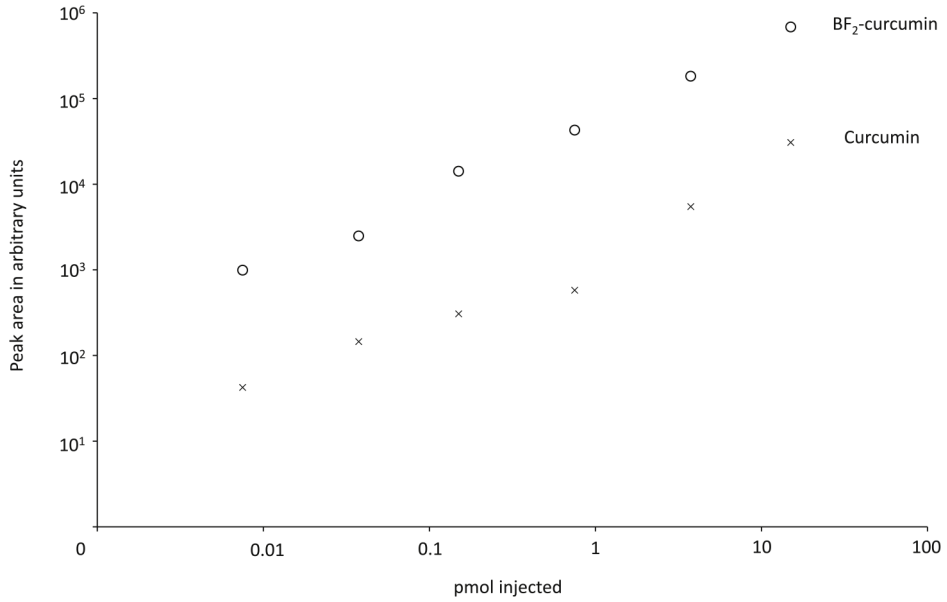


Fig. 4.

Peak areas for curcumin (diamonds, *m/z* 368 parent →*m/z* 217 fragment) and BF2-curcumin (circles, *m/z* 415 parent → *m/z* 400 fragment) using previously optimized LC/MS conditions for each. The data was obtained from plasma samples spiked with curcumin and processed as described in Methods and Results & Discussion. The peaks areas for the BF_2–C signals were on average 28-fold greater than the peak areas for the C signals.

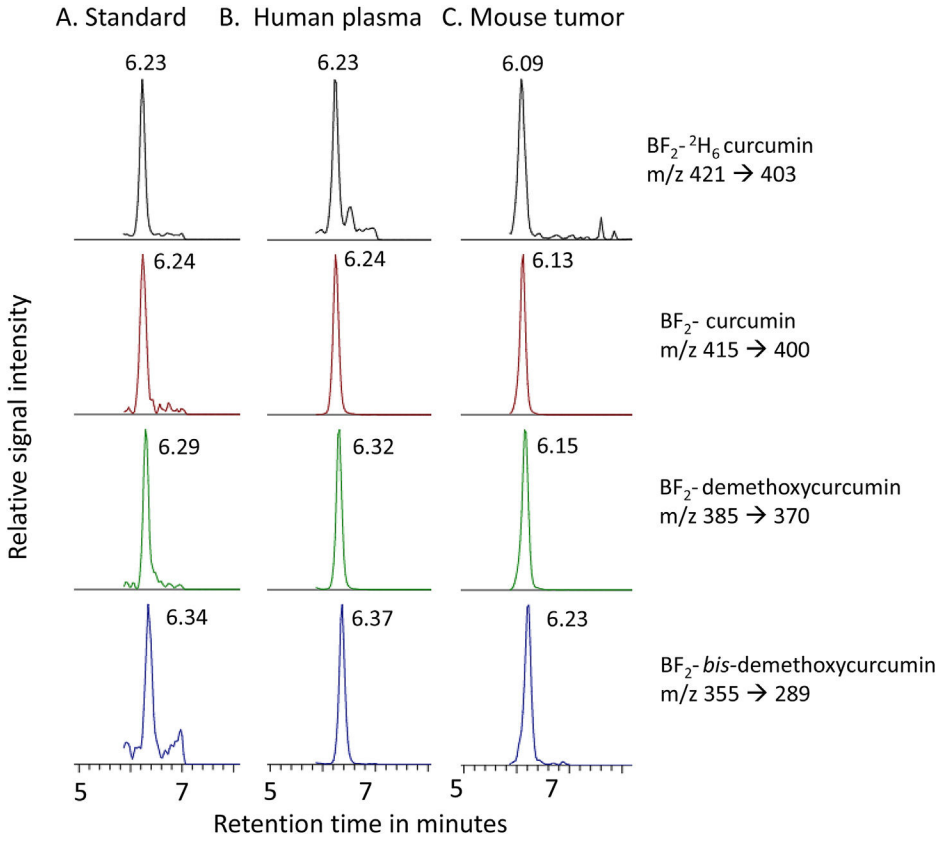


Fig. 5.

Select regions of the reconstructed ion chromatograms for the curcuminoids in: A, human plasma spiked with standards at the practical LoQ (0.5 nM for C, DMC, BDMC, equivalent to 15 fmol of each compound injected assuming 100% recovery; B, Extract of Day 17 plasma from a volunteer who orally consumed four tablets of a commercially available over-the-counter curcuminoid formulation (Longvida®, 260 mg curcuminoids per 1000 mg tablet) for 17 consecutive days; C, Extract from 83.8 mg of tumor from a mouse that had been administered PHRAD 129 turmeric preparation.

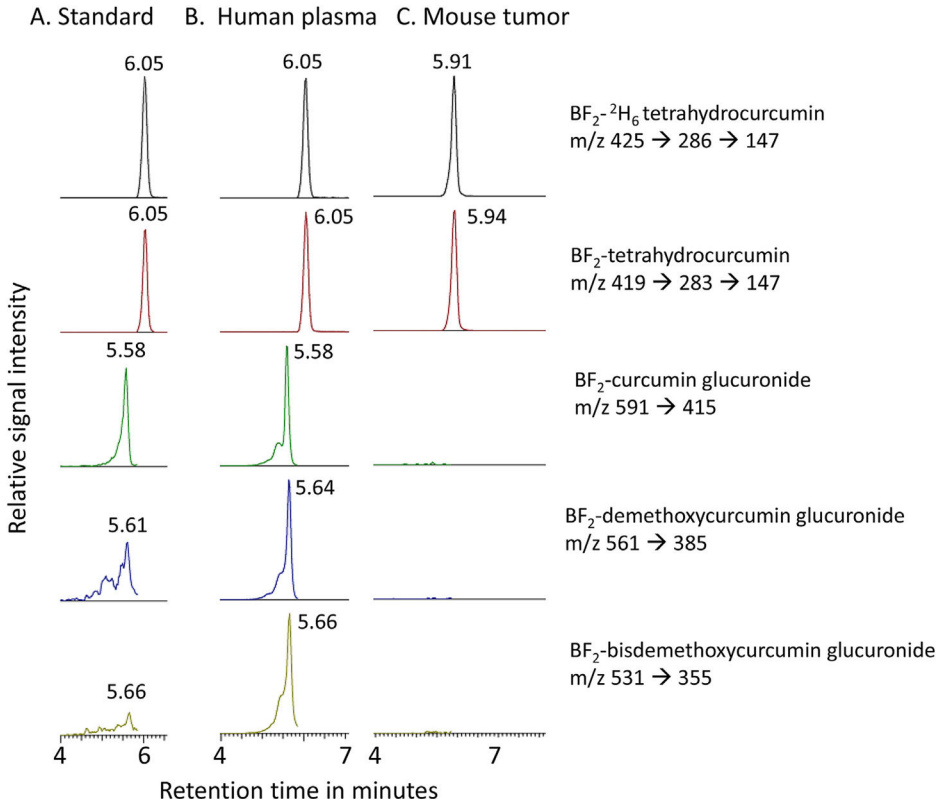


Fig. 6.

Select regions of the reconstructed ion chromatograms for the curcuminoid metabolites in: A, human plasma spiked with standards at the practical LoQ (5 nM for TC and CG, equivalent to 150 fmol of each compound injected assuming 100% recovery; B, human plasma from a volunteer who orally consumed four tablets of a commercially available over-the-counter curcuminoid formulation (Longvida®, 260 mg curcuminoids per 1000 mg tablet) daily for 17 consecutive days; C, Extract from 83.8 mg of tumor from a mouse that had been administered PHRAD 129 turmeric preparation.

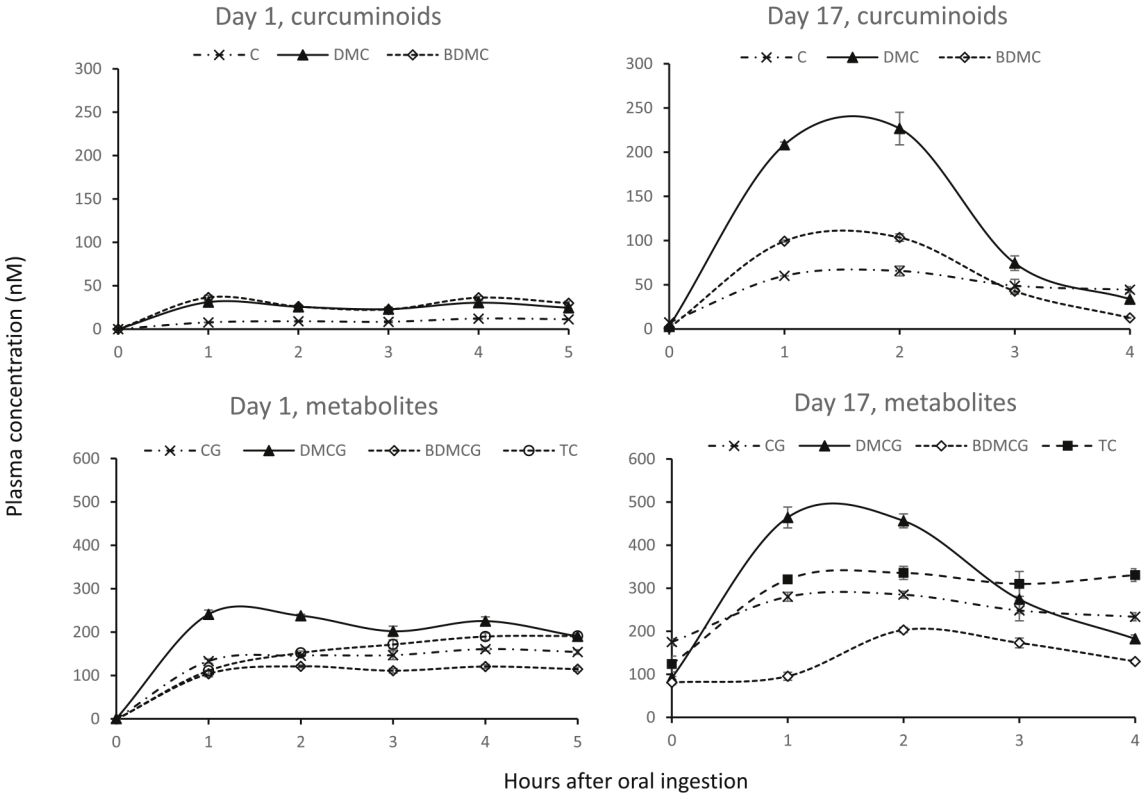


Fig. 7.

Temporal profiles of the concentration of curcuminoids [curcumin (C), demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC; left panels] and curcumin metabolites [tetrahydrocyrcumin (TC) and C, DMC and BDMC glucuronides (G); right panels] on Day 1 (upper panels) and Day 17 (lower panels) in the plasma of the volunteer who orally consumed four tablets of over-the-counter curcuminoid formulation (Longvida®, 260 mg curcuminoids per 1000 mg tablet) for 17 consecutive days. Blood was collected at 0, 1, 2, 3, 4, and 5 h on each of Days 1 and 17. Data points are the mean ± standard error of the mean from triplicate aliquots analyzed for each time point

Table 1

Summary of the mass spectral characteristics of the BF_2-curcuminoid derivatives.

Compound	Elemental composition of BF_2-derivative	Observed $[M - H]^-$ ion (*m/z*)	Observed MS^2 and MS^3 transitions	Mass lost (Da)	Assigned loss
Curcumin (C)	$C_{21}H_{19}O_6BF_2$	415	415 → 400	15	CH_3
$^2H_6$-Curcumin (D6C)	$C_{21}H_{13}D_6O_6BF_2$	421	421 → 403	18	C^2H_3
Demethoxycurcumin (DMC)	$C_{20}H_{17}O_5BF_2$	385	385 → 370	15	CH_3
Bisdemethoxycurcumin (BDMC)	$C_{19}H_{15}O_4BF_2$	355	355 → 289	66	BF_2OH
Curcumin β-D-glucuronide (CG)	$C_{27}H_{27}O_{12}BF_2$	591	591 → 415	176	glucuronic acid
Tetrahydrocurcumin (TC)	$C_{21}H_{23}O_6BF_2$	419	419 → 283 → 147	136, 136	$C_8H_8O_2$
$^2H_6$-Tetrahydrocurcumin (D6TC)	$C_{21}H_{17}D_6O_6BF_2$	425	425 → 286 → 147	139, 139	$C_8H_5{}^2H_3O_2$
Demethoxycurcumin glucuronide (DMCG)	$C_{26}H_{25}O_{11}BF_2$	561	561 → 385	176	glucuronic acid
Bisdemethoxycurcumin glucuronide (BDMCG)	$C_{25}H_{23}O_{10}BF_2$	531	531 → 355	176	glucuronic acid

Table 2

Summary of curcuminoid recovery efficiencies from human plasma (upper panel) and mouse cerebellar tissue (lower panel). Experiments were performed as described in Methods and Results & Discussion. The final concentrations of C, DMC, and BDMC in QC-low, QC-medium, QC-high samples were 0.5, 2.5, and 25 nM, respectively, while for TC and CG, the final concentrations were 5, 25, and 100 nM, respectively.

Recovery from human plasma	**QC-low**		**QC-medium**		**QC-high**	
	concentration (nM)	**% recovery**	**concentration (nM)**	**% recovery**	**concentration (nM)**	**% recovery**
Curcumin	0.5	101.8	2.5	87.4	25	88.0
Demethoxycurcumin	0.5	95.1	2.5	89.1	25	87.8
Bisdemethoxycurcumin	0.5	101.8	2.5	86.6	25	92.0
Tetrahydrocurcumin	5	117.9	25	104.2	100	101.3
Curcumin β-D-glucuronide	5	98.2	25	82.0	100	85.3

Recovery from mouse cerebellum

	Spiked amount	**% recovery**
Curcumin	2.5 pmol/30 mg tissue	80.8
Demethoxycurcumin	2.5 pmol/30 mg tissue	97.6
Bisdemethoxycurcumin	2.5 pmol/30 mg tissue	87.4
Tetrahydroxycurcumin	2.5 pmol/30 mg tissue	97.6
Curcumin β-D-glucuronide	2.5 pmol/30 mg tissue	10.8

Table 3

Summary of accuracy and precision validation analysis in human plasma.

Plasma accuracy and precision												
	QC-low				**QC-medium**				**QC-high**			
	Nominal (nM)	**Calculated (nM)**	**% accuracy**	**% CV**	**Nominal (nM)**	**Calculated (nM)**	**% accuracy**	**% CV**	**Nominal (nM)**	**Calculated (nM)**	**% accuracy**	**% CV**
Curcumin	0.5	0.49 ± 0.03	98.41	10.54	2.5	2.61 ± 0.06	109.19	3.88	25	26.42 ± 0.07	105.69	0.44
Demethoxycurcumin	0.5	0.44 ± 0.03	88.9	13.22	2.5	2.56 ± 0.19	102.21	8.4	25	25.15 ± 0.48	100.60	3.33
Bisdemethoxycurcumin	0.5	0.50 ± 0.04	99.24	13.48	2.5	2.36 ± 0.19	94.39	14.21	25	25.98 ± 1.39	103.91	9.25
Tetrahydrocurcumin	5	6.84 ± 1.41	136.78	35.68	25	32.06 ± 2.76	128.22	14.93	100	148.91 ± 10.98	148.91	12.78
Curcumin β-D-glucuronide	5	5.20 ± 0.25	104.06	8.23	25	23.61 ± 0.85	94.43	6.27	100	96.14 ± 6.36	96.14	11.46