# Highly sensitive HPLC method for estimation of total or individual curcuminoids in *Curcuma* cultivars and commercial turmeric powders

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**The action of turmeric depends on three curcuminoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin, whose distribution is highly varied among cultivars. A sensitive method for estimation of all three curcuminoids is essential for quality control. We developed a HPLC-MS method with lowest limits of detection and quantification of curcuminoids. Mass spectrometry (MS) authenticated the identity of curcuminoids. Principal component analysis of curcuminoids established the high variation among the selected seven cultivars of *Curcuma,* as well as commercial powders. We suggest that our HPLC method can be used for quality control of turmeric.**

**Keywords:** *Curcuma*, curcuminoids, cultivars, metabolomics, variation.

TURMERIC is a rhizomatous herbaceous perennial plant, belonging to the ginger family, Zingiberaceae. It is native to tropical South Asian countries, but is widely cultivated in the tropical and subtropical regions of the world. There are about 120 known species of *Curcuma*[1] within the family of Zingiberaceae. Studies have been conducted on various *Curcuma* species, particularly *C. longa* (turmeric), *C. aromatica* (wild turmeric), *C. xanthorrhiza* (Japanese turmeric), *C. amada* and *C. kwangsiensis*. *Curcuma longa* has been commonly used as spice and medicine in Asia, including India. In Ayurveda medicine and traditional Chinese medicine, turmeric is used as anti-inflammatory agent and also as aspirant, cordeal, emenagogue, astringent and diuretic[2,3]. Wild turmeric (*C. aromatica* Salisb., commonly called as Kasthurimanjal or yujin) is cultivated in some regions of India and China[4,5].

The colouring and pharmacological activities of turmeric powder are attributed to the presence of curcuminoid pigments belonging to the class of diaryl-heptanoids. Three major curcuminoids identified are: curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, CU), demethoxycurcumin (4-hydroxy-cinnamoyl-(feruloyl) methane, DMC) and bis-demethoxycurcumin (bis(4-hydroxycinnamoylmethane), BDMC). Curcuminoids exhibit a wide range of biological activities. Though most of the studies are focused on pharmacological/biological activities of CU[2,6], a few reports point out the effectiveness of DMC and BDMC. All three are known for their potency in suppression of tumour necrosis factor (TNF)-induced by nuclear factor-$\kappa$B (NF-$\kappa$B) activation, which modulates inflammatory state in mammalian cells. DMC has shown its pharmacological effect in inhibiting proliferation of breast cancer cells[7] and vascular smooth muscle cell migration[8]. Another study showed BDMC as a promising compound in the treatment of type-2 diabetes[9]. These studies suggest that not only CU, but also other curcuminoids are important.

Cultivars of *C. longa* and other species are highly variable in their curcuminoid content. Studies on local cultivars need to be conducted to determine curcuminoids and identify high-yielding varieties. In addition to *C. longa*, *C. aromatica* is an economically important species of genus *Curcuma*, and used in medicine and toiletry[3]. Studies on rhizomes of *C. aromatica* showed its pharmacological effect[10], but there are only few reports on quantitation of curcuminoids in *C. aromatica*[11,12].

In view of the growing importance of turmeric in food and medicine, it is imperative that we evolve measures to quantify all three curcuminoids. Several methods using TLC[13], two-dimensional TLC[14], HPTLC-densitometry[15], HPLC[16] and LC-MS[17] have been employed for qualitative and quantitative analysis of curcuminoids content in *Curcuma* species. However, several of these studies exhibited high values for limit of detection (LOD) and limit of quantification (LOQ) of curcuminoids[18]. Due to the great importance of curcuminoids in food and medicine, there

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is need for effective and sensitive methods to monitor all three curcuminoids.

A highly sensitive HPLC method was employed for curcuminoid quantification and the identity of three curcuminoids was validated, by mass spectrometry and use of standard compounds. Quantitative analysis of three major curcuminoids and their relative distribution in five cultivars of *Curcuma longa* and two cultivars of *C. aromatica* was performed by HPLC. A similar study was extended to commercial turmeric powders – seven branded and two non-branded ones. This article is the first comprehensive report on curcuminoids in the selected five cultivars of *C. longa* and two cultivars of *C. aromatica* and also commercial turmeric powders, all from India, though there are reports on other cultivars of *C. longa*[19,20] and commercial turmeric extracts[18]. Our modified gradient HPLC method adapted from Jiang *et al.*[21], is highly sensitive and yielded very low LOD and LOQ values. In addition, our modified method consumed much less solvent compared to earlier published methods[18,21].

## Experimental

### *Materials and reagents*

LC-MS grade methanol, water and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium formate and formic acid were purchased from Sigma-Aldrich, India. The reference compounds, curcumin (CU, 97.2%), demethoxycurcumin (DMC, 95.0%), and bisdemethoxycurcumin (BDMC, 99.9%) were obtained from ChromaDex (California, USA). Fresh rhizomes of four cultivars of *Curcuma longa* (cvs. Duggirala Red, Prathibha, Salem and Suguna) and two cultivars of *C. aromatica* (cvs. Kasturi Araku and Kasturi Avidi), were collected from Turmeric Research Station, Kammarpally of Telangana State, India. Alleppey Supreme cultivar of *C. longa* was obtained from Indian Institute of Spices Research, Marikunnu (IISR) Kozhikode, Kerala, India. All the rhizomes were cryopreserved at –80°C until analysis. Five branded (BC-1 to BC-5) and two non-branded (LNB-1 and LNB-2) commercial turmeric powders were procured from the local markets of Andhra Pradesh, while two branded (BC-6 and BC-7) commercial turmeric powders were from Kerala.

### *Preparation of samples and standards*

Fresh turmeric rhizomes of *Curcuma longa* cvs. Alleppey Supreme, Duggirala Red. Prathibha, Salem, Suguna and *C. aromatica* cvs. Kasturi Araku and Kasturi Avidi were ground using mortar and pestle in presence of liquid nitrogen. The ground material was soaked in LC grade methanol (500 mg fresh weight/ml), sonicated for 30 min and extracts were centrifuged at 1500 *g* for 25 min. Finally, the supernatant was filtered through 0.2 µm PTFE membrane filter. Filtered supernatant was diluted 10 times and 0.5, 1 and 2 µl aliquots were injected for HPLC analysis. In case of commercial turmeric powders, 5 mg of turmeric powder was extracted with 500 µl of methanol and rest of the procedure was the same as described previously, until the sample was filtered. Then, the supernatant was diluted four times before injecting 2 µl for HPLC analysis. Filtered extracts were kept at –20°C until analysis. Dry weight of rhizomes of each cultivar of *C. longa* and *C. aromatica* was determined as follows: rhizomes were cooked in boiling water, blotted to remove excess water, followed by oven drying at 55°C for 7–8 days. The amount of curcuminoid content expressed on basis of dry weight. CU, DMC and BDMC were accurately weighed and dissolved in methanol, and then stored at –20°C until HPLC analysis. The concentration of the three compounds in stock solution was 1 mg/ml. The recovery of curcuminoids was checked as follows: an aliquot of 20 µl of CU (1 µg/µl) was spiked with 5 mg of commercial turmeric powder, and then extracted with 480 µl of methanol. Rest of the extraction procedure was the same as described for fresh rhizome. Each sample of 2 µl was injected in duplicate for HPLC analysis. Samples were kept at –20°C until analysis.

### *HPLC and HPLC-QTOF-MS conditions*

An Agilent 6520 HPLC system including a binary pump and a UV-Vis detector coupled with QTOFMS was used for qualitative and quantitative analyses of curcuminoids from methanolic rhizome extracts of *Curcuma*. The separation of compounds methodology was adapted from Jiang *et al.*, with modifications as described. Zorbax Eclipse XDB-C18 reverse phase column with dimensions of 4.6 mm × 50 mm, 1.8 µ reverse column was used at 40°C; The mobile phase was a programmed gradient, consisting of (a) ammonium formate (5 mM in 0.1% formic acid, pH adjusted to 3.00) and (b) acetonitrile (100%, v/v). The programmed gradient was 0–20% (b) in 0–2 min, 20–100% (b) in 2–28 min, 100% (b) in 28–35 min, 100–20% (b) in 35–38 min. The mobile phase flow rate was 0.2 ml/min. The diode array detector (DAD) was monitored at 425 nm. q-TOF-MS (Agilent Santa Clara, CA) was equipped with an electron spray ionization (ESI) interface as the ion source was used for the identification of compounds in all *Curcuma* cultivars and commercial turmeric powders. The acquisition parameters were: drying N2 temperature, 350°C, 8 l/min; nebulizer pressure 40 psi; HV capillary 4000 V; skimmer 65.0 V.

### *Qualitative and quantitative analysis*

*Detection, identification and quantitation and recovery of curcuminoids:* Three major curcuminoids (CU, DMC

and BDMC) were identified in five cultivars of *Curcuma longa* and two cultivars of *C. aromatica* based on their retention time and mass-fragmentation spectra. A similar approach was achieved for seven branded and two non-branded turmeric powders. The contents of these compounds were determined using peak areas obtained from HPLC-DAD chromatograms taken at 425 nm. The retention time of BDMC, DMC and CU was 20.8, 21.3 and 21.9 min respectively (Figure 1). Curcuminoids in all samples were quantitated using standard regression equation generated using reference. MS analysis revealed that no other compound(s) were eluted at the retention times of BDMC, DMC and CU. The (+)-ESI mass spectra gave characteristic molecular ions of CU ($[M + H]^+$ ion at *m/z* 369), DMC ($[M + H]^+$ ion at *m/z* 339), and BDMC ($[M + H]^+$ ion at *m/z* 309).

To obtain the calibration curve of each of the curcuminoids, working solution of seven concentrations containing CU, DMC or BDMC were analysed. The calibration curves were established by plotting peak areas versus the concentration of each curcuminoid. In the regression equation $y = mx + c$, $x$ refers to the concentration of pure curcuminoids (μg/ml), $y$ the peak area, $m$ and $c$ represent the slope and $y$ intercept, respectively (see Supplementary Figure S1 online). The calculation method of LOD and LOQ was based on the standard deviation (SD) of the response and the slope ($m$) of the calibration curve according to formula LOD = 3.3 (SD/$m$) and LOQ = 10 (SD/$m$) respectively[18]. Calibration curves of the three curcuminoids showed good linearity in relatively wide dynamic ranges, averaged HPLC peak areas in mV yielded for each curcuminoid concentrations of 3.125, 6.25, 12.5, 25, 50, 100 and 200 ng were in the following order for BDMC: 44.51, 94.2, 214.43, 439.75, 820.41, 1603.78 and 3283; DMC: 59.07, 124.34, 249.2, 495.5, 982.37, 1927.9 and 3546.28; CU: 54.74, 114.39, 236.71, 485.95, 981.63, 1897.98 and 3665.85 (see Supplementary Figure S1 online).

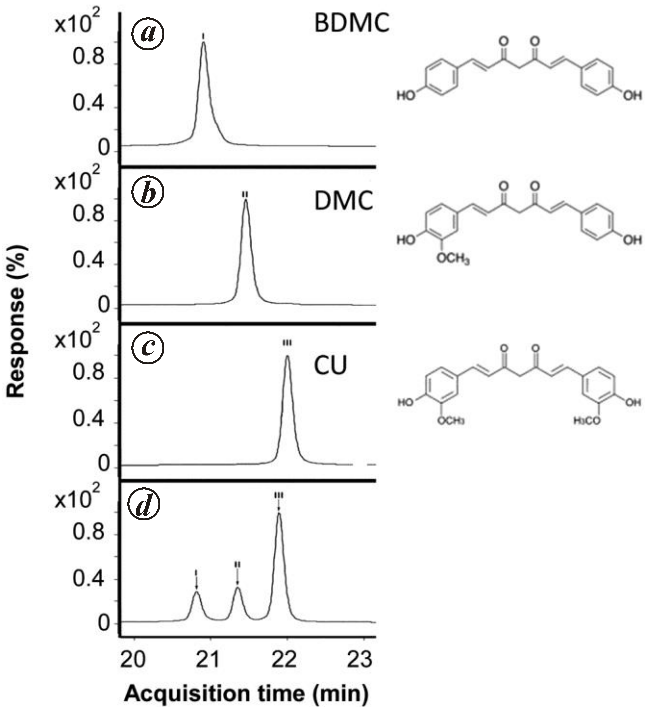


**Figure 1.** Pure curcuminoids of BDMC, DMC and CU were subjected to HPLC column chromatography and their elution profile was recorded. Representative HPLC-DAD chromatograms of curcuminoids: ***a***, standard BDMC; ***b***, standard DMC; ***c***, standard CU, corresponding structures of these curcumionoids were shown (source: Wikipedia); Retention times of these curcuminoids were 20.8, 21.3 and 21.9 min respectively. ***d***, Methanolic extract of rhizomes of *C. longa* cv. Alleppey Supreme having all three compounds of which peaks labelled as I, II and III corresponding to BDMC, DMC and CU respectively.

Calibration curve for all three curcuminoids showed good linearity ($r^2 > 0.998$) through the selected range of concentrations of 3.125–200 ng (see Supplementary Figure S1 online). Recovery test was used to evaluate the accuracy in quantitation of CU in commercial powders of this method. An aliquot of 20 μl of pure compound (stock of 1 μg/μl) of CU was spiked to approximately 5 mg of the commercial turmeric powder, and then extracted with 480 μl of methanol and analysed as described for other samples (see Supplementary Table S2 online). Estimated amount of CU in spiked commercial turmeric powder sample in comparison with respective unspiked samples was used to calculate percent recovery.

*Data analysis by principal component analysis:* Principal component analysis (PCA) helps in comparing more than two datasets against each other[22]. PCA analysis of all three curcuminoid contents of seven cultivars and nine commercial turmeric powders was done using 'Metaboanalyst' (ver. 3) online software. Curcumin content was taken as reference compound.

## Results and discussion

### *Quantification of three curcuminoids in cultivars of Curcuma species*

The curcuminoids in extracts of rhizomes were separated well by HPLC. The HPLC-DAD chromatogram (425 nm) of rhizomes of all cultivars (example shown of cv. Alleppey Supreme *C. longa*), revealed the conspicuous presence of three curcuminoids (Figure 1). The three standard pure curcuminoids, BDMC, DMC and CU eluted at 20.8, 21.3 and 21.9 min respectively, gave distinct peaks at 425 nm (Figure 1). Their corresponding structures (source: Wikipedia) are shown in Figure 1. The identity of each of these curcuminoids was established from total ion current (TIC) chromatogram collected in (+)-ESILC-MS mode of three standard curcuminoids and of all cultivars of *Curcuma* species is shown in Figure 2 *a* and *b* respectively. Further MS/MS analysis exhibited characteristic

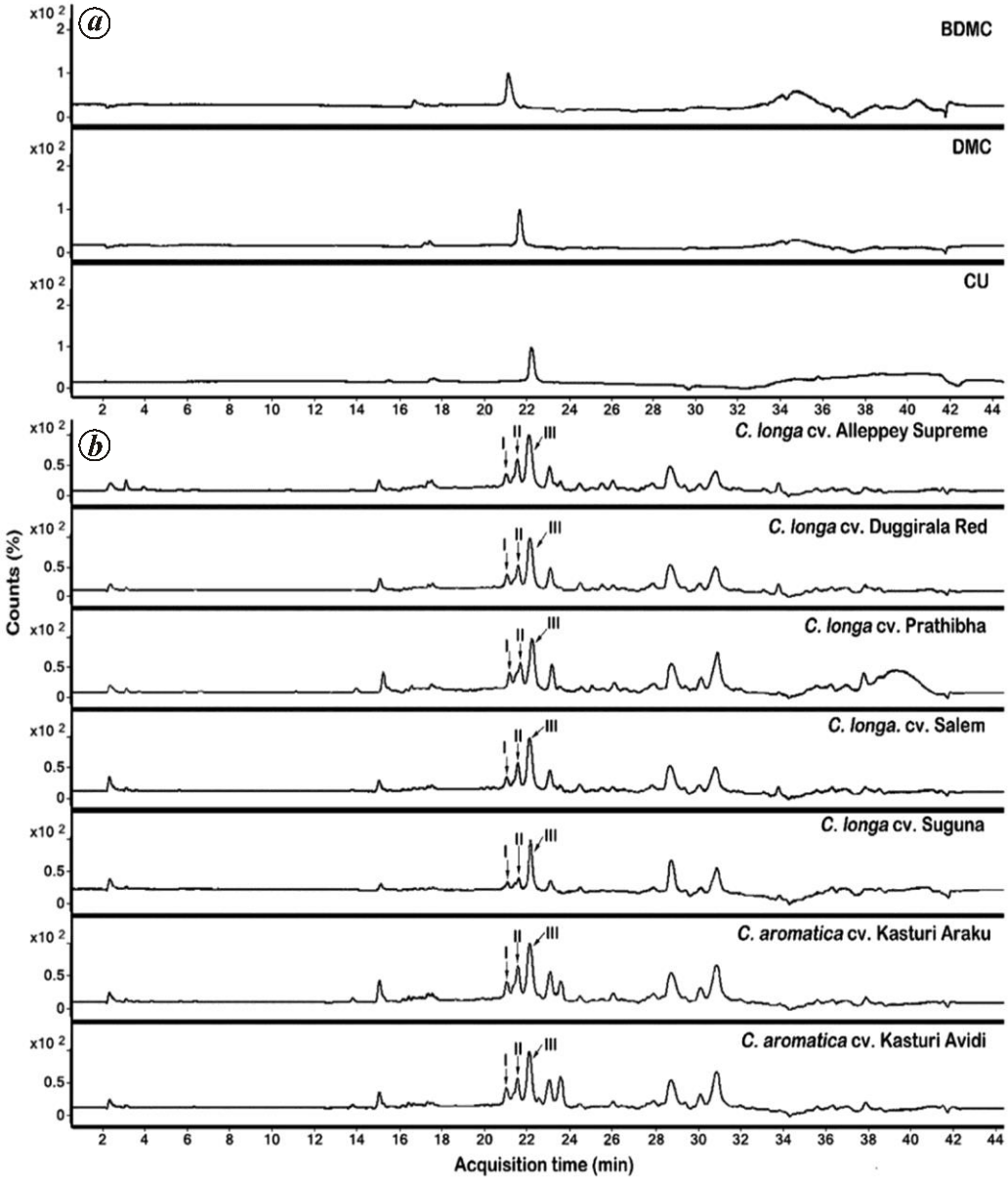


**Figure 2.** Representative total ion current (TIC) chromatograms from positive ion (+)-ESI-HPLC-MS: (***a***) three standard curcuminoids, BDMC, DMC and CU of masses respectively of 308.1, 338.1 and 368.1 and (***b***) rhizomes of seven cultivars of *Curcuma*: five of *C. longa* and two of *C. aromatica*. Peaks with labels I, II and III are BDMC, DMC and CU respectively confirmed by retention times and masses of corresponding standard pure curcuminoids.

**Table 1.** BDMC, DMC and CU curcuminoid content of rhizomes from seven cultivars of *Curcuma*, determined by subjecting their methanolic extracts to HPLC-DAD analysis (at 425 nm)

| | BDMC | DMC | CU | Total |
|---|---|---|---|---|
| Cultivars (species) | mg/g dry weight | | | |
| Alleppey supreme (*C. longa*) | 7.3 ± 0.52 | 7.1 ± 0.35 | 21.6 ± 0.40 | 36.0 |
| Duggirala red (*C. longa*) | 4.5 ± 0.25 | 3.3 ± 0.09 | 9.9 ± 0.15 | 17.7 |
| Prathibha (*C. longa*) | 3.7 ± 0.18 | 2.0 ± 0.14 | 3.9 ± 0.18 | 9.6 |
| Salem (*C. longa*) | 2.9 ± 0.13 | 3.0 ± 0.14 | 7.2 ± 0.20 | 13.1 |
| Suguna (*C. longa*) | 5.2 ± 0.04 | 3.2 ± 0.17 | 7.0 ± 0.14 | 15.4 |
| Kasturi araku (*C. aromatica*) | 5.4 ± 0.27 | 4.3 ± 0.18 | 8.3 ± 0.15 | 18.0 |
| Kasturi avidi (*C. aromatica*) | 6.2 ± 0.13 | 3.6 ± 0.11 | 7.6 ± 0.12 | 17.4 |

Values (mg/g dry weight) shown are average of minimum of three independent experiments.

fragmentation pattern of curcuminoids identical to previous report: *m/z* 369, 285, 245, 219,177 for CU; *m/z* 339, 255, 245, 219, 177, 147, 145 for DMC and *m/z* 309, 225, 189, 177, 147 for BDMC[21]. Representative spectra for (+)-ESI-MS and (+)-ESI-MS/MS fractions of three curcuminoids are shown in supplementary data (Figures 2 *a* and 3).

Among all the cultivars, the amount of total curcuminoids varied considerably (Table 1). Seven cultivars of *Curcuma longa* and *C. aromatica* contained percentages of curcuminoids BDMC, DMC and CU, respectively based on their rhizome dry weight: namely Alleppey Supreme: 0.73%, 0.71%, 2.16%; Duggirala Red: 0.45%, 0.33%, 0.99%; Prathibha: 0.37%, 0.2%, 0.39%; Salem: 0.29%, 0.3%, 0.72%; Suguna: 0.52%, 0.32%, 0.7%; Kasturi Araku: 0.54%, 0.43%, 0.83 and Kasturi Avidi: 0.62%, 0.36%, 0.76%. By comparison, Cultivar Alleppey Supreme (*C. longa*) contained highest amounts of curcuminoids (36 mg/g dry weight), followed by cv. Duggirala Red (18 mg/g dry weight), while lowest amount was found in *C. longa* cv. Prathibha (10 mg/g dry weight). The amounts of curcuminoids varied significantly among the cultivars of *C. longa*, although they were grown in the same region (cvs. Duggirala Red, Prathibha, Salem and Suguna). Cultivars of *C. aromatica* had about the same amount of total curcuminoid content as that of *C. longa* cv. Duggirala Red. *C. aromatica* cvs. Kasturi Araku and Kasturi Avidi had 18 mg/g dry weight and 17 mg/g dry weight of total curcuminoids respectively. Amongst different cultivars of *C. longa*, the content of each curcuminoid also varied remarkably, 4 to 22 mg/g dry weight for CU, 2 to 7 mg/g dry weight for DMC, and 3 to 7 mg/g dry weight for BDMC. In case of *C. aromatica* cv. Kasturi Araku had more CU (8 mg/g dry weight) and DMC (4 mg/g dry weight). Higher amount of CU (7.6 mg/g dry weight) and BDMC (6.2 mg/g dry weight) were found in cv. Kasturi Avidi. All values are shown in Table 1.

### *Importance of DMC and BDMC, besides CU*

Out of three major curcuminoids (CU, DMC and BDMC), the levels of CU were the highest in all the cultivars. In literature, most of the pharmacological activities were assayed with either CU alone or mixture of all three curcuminoids[1,2]. However, the importance of DMC and BDMC, independent of CU is being recognized[8] as some reports show higher effectiveness of DMC and BDMC than CU[9,23]. Therefore, the present study emphasized the equal importance of analyses of all the three (CU, DMC and BDMC) curcuminoids in different cultivars of *Curcuma*. Further, our results point out the conspicuous variation in the relative distribution of the three curcuminoids (CU, BDMC and DMC) in rhizomes of seven cultivars. We therefore suggest that not only the CU levels, but also the content of other curcuminoids should be considered while selecting cultivars for turmeric production.

### *Curcuma aromatica as promising as C. longa*

Identification of high curcuminoid yielding species and cultivars of *Curcuma*, is important. In view of this, our attempts to determine the curcuminoid content in two cultivars of *C. aromatica* besides the five cultivars of *C. longa* are of significance. Our study reveals that *C. longa* cv. Alleppey Supreme from Kerala had highest curcuminoid content among all the seven selected (36 mg/g dry weight) cultivars of *Curcuma*. Among cultivars from Andhra Pradesh, Duggirala Red of *C. longa* had high curcuminoid (18 mg/g dry weight) content. Thus, our results endorse the aptness of farmers' practice – the cultivation of high curcuminoid yielding cultivars of *C. longa* – cv. Alleppey Supreme in Kerala and *C. longa*, cv. Duggirala Red and *C. aromatica* cvs. Kasturi Araku and Kasturi Avidi in Andhra Pradesh. *C. aromatica* is considered important for its medicinal and cosmetic value in Indian traditional medicine, but reports on three curcuminoids in Indian cultivars of *C. aromatica* have been scanty[12,24]. Only one report[12] described presence/levels of CU, DMC and BDMC by HPLC method in three Indian varieties of *C. aromatica*. We suggest that *C. aromatica* (cvs. Kasturi Araku and Kasturi Avidi) can be used as alternative sources for production of curcuminoids (having up to 18 mg/g dry weight).

### *Quantitative analysis of curcuminoids in commercial turmeric powders*

The established validated method was applied to identify three major curcuminoids (CU, DMC and BDMC) in seven branded and two non-branded commercial turmeric powders by HPLC method on dry weight basis. HPLC-UV method was applied for quantitation of curcuminoids. Their MS fractions were elucidated by using HPLC-q-TOF-MS and are confirmed by q-TOF-MS/MS analysis. These samples varied in total curcuminoid content as well as in amount of individual curcuminoid. The highest amount of total curcuminoid content was found in branded turmeric powder, BC-4 (28 mg/g dry weight), and the lowest amount of 11 mg/g dry weight was found in local non-branded turmeric powder LNB-2 (Table 2).

### *Evaluation of Curcuma cultivar variation based on PCA clustering*

Total curcuminoid content and internal ratios among CU, BDMC and DMC to their total curcuminoid content varied among the seven cultivars of *Curcuma.* They clustered into three groups. In PCA analysis, *C. longa* cv. Alleppey Supreme had highest total curcuminoid content and also had highest content of all three curcuminoids independently. This cultivar clustered as a separate entity (cluster I, Figure 3). *C. longa* cv. Prathibha had lowest

**Table 2.** Content of three major curcumionoids, BDMC, DMC and CU in commercial turmeric powders, determined by subjecting their metanolic extracts to HPLC-DAD analysis (at 425 nm)

| | BDMC | DMC | CU | Total |
|---|---|---|---|---|
| Commercial powder | mg/g dry weight | | | |
| BC-1 | 4.8 ± 0.07 | 4.5 ± 0.02 | 15.5 ± 0.75 | 24.8 |
| BC-2 | 3.9 ± 0.05 | 3.9 ± 0.04 | 12.5 ± 0.07 | 20.3 |
| BC-3 | 4.9 ± 0.24 | 5.0 ± 0.09 | 17.7 ± 0.67 | 27.6 |
| BC-4 | 5.4 ± 0.05 | 5.3 ± 0.06 | 17.3 ± 0.05 | 28.0 |
| BC-5 | 4.3 ± 0.31 | 4.2 ± 0.02 | 16.3 ± 0.76 | 24.8 |
| BC-6 | 3.3 ± 0.13 | 2.9 ± 0.12 | 7.6 ± 0.11 | 13.8 |
| BC-7 | 5.3 ± 0.07 | 5.3 ± 0.07 | 11.2 ± 0.01 | 21.8 |
| LNB-1 | 2.5 ± 0.02 | 2.7 ± 0.01 | 11.9 ± 0.10 | 17.1 |
| LNB-2 | 1.7 ± 0.06 | 1.4 ± 0.01 | 7.4 ± 0.26 | 10.5 |

Values (mg/g dry weight) shown are average of minimum two independent experiments.

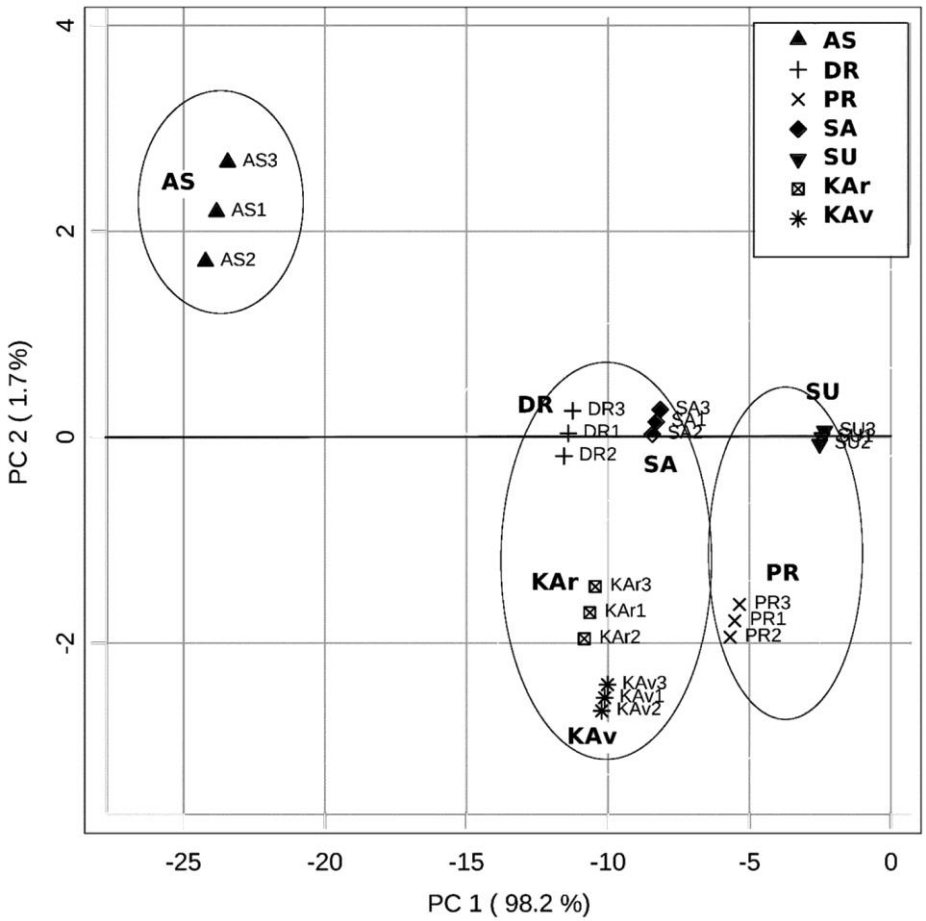


**Figure 3.** Principal component analysis (PCA) plot of the cultivars of *C. longa* and *C. aromatica* based on the amount of three curcuminoids, BDMC, DMC and CU, in their rhizomes quantitated using HPLC-DAD analysis. Cultivars of *C. longa* – AS, Alleppey Supreme; DR, Duggirala Red, PR, Prathibha; SA, Salem; SU, Suguna. Cultivars of *C. aromatica*; Kar, Kasturi Araku and KAv, Kasturi Avidi.

total curcuminoid content. Alleppey Supreme plotted at top left corner, whereas, Prathibha occupied lower right corner of the PCA plot. Cultivars of *C. longa* cvs. Duggirala Red, Salem and cultivars of *C. aromatica* cvs. Kasturi Araku and Kasturi Avidi grouped into cluster II. Cultivars of *C. longa* cvs. Suguna and Prathibha grouped into cluster III. In cluster III, Suguna and Prathibha had varied amounts of total curcuminoids, but the internal ratio among curcuminoids is closely related. In cluster II, all four cultivars greatly differed from each other in the ratios.

**Table 3.** Comparison of HPLC methodology parameters used for curcuminoid separation and resultant LOD and LOQ values (CU, DMC and BDMC) of present study versus literature

| Column/flow rate/ wavelength | Detector/gradient/ isocratic | Injected amount of curcuminoid | LOD (ng/ml) | | | LOQ (ng/ml) | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | CU | DMC | BDMC | CU | DMC | BDMC | |
| Zorbax eclipse XDB C18, (50 × 4.6 mm,1.8 µ) 0.2 ml/min 425 nm | DAD (A) 5 mM ammonium formate in 0.1% formic acid and (B) 100% (v/v) ACN; 0–20% B in 0–2 min; 20–100% B in 2–28 min; 100% B in 28–35 min; 100–20% B in 35–38 min | 3–200 ng | 0.06 | 0.10 | 0.36 | 0.20 | 0.34 | 1.20 | Present study |
| Vydac®, RP-18 (250 mm × 4.6 mm, 5 µm) 1.5 ml/min 420 nm | UV–Vis Acetonitrile: 0.1% trifluroacetic acid (TFA) (50 : 50 v/v), (adjusted to pH 3.0 with ammonia) mixture | 100–600 ng/ml | 28 | 32 | 22 | 85 | 97 | 66 | 16 |
| Chromolith merck (100 × 4.6 mm, 2 µm) 1 ml/min 425 nm | UV–Vis Water–ACN–glacial acetic acid (60 : 40 : 1, v/v/v) mixture | 0.5–100 µg/ml | 50 | 50 | 50 | 100 | 100 | 100 | 29 |
| Alltect Alltima C18 (150 × 4.6 mm, 5 µm) 2 ml/min 425 nm | UV–Vis Acetonitrile and 2% acetic acid (40:60,v/v) mixture | CU (10–60 µg/ml) DMC (4–24 µg/ml) BDMC (0.5–3.0 µg/ml) | 900 | 840 | 80 | 2730 | 2530 | 230 | 18 |

### *Quality assessment of commercial turmeric powders based on PCA clustering*

Our report provides a quantitative analysis of curcuminoids, their relative quantities in Indian commercial turmeric powders. BC-1 to BC-6 had similar levels of total curcuminoid content as well as CU, DMC and BDMC. BC-7 had lowest level of CU among all the commercial powders, but had relatively high amounts of BDMC and DMC. LNB-1 and LNB-2 had very low levels of DMC, BDMC as well as CU. In turmeric powders, a minimum of 2% CU is suggested by the Bureau of Indian Standards[25] (BIS 2010). In our study, six branded turmeric powders had more than 2% CU content, while one brand (BC-6) and both local non-branded turmeric powders fall below this standard (Table 2). Thus, PCA analysis clearly distinguishesd common turmeric powders, which did not meet the quality standard (Figure 4). Further studies are required to understand the significance and possible exploitation of variations in the ratios of the three curcuminoids.

### *HPLC analysis of curcuminoids: a good tool to distinguish cultivars and ascertain the quality of commercial powders*

Although the content of curcuminoid is important for export market of turmeric, many countries such as Germany, Netherlands, Spain, UK and USA have not specified minimum quantity of curcuminoids[26,27]. Since turmeric powder is used as spice and medicine around the world, major precautions must be undertaken during not only its preparation, but also selecting rhizomes and using superior cultivars. The present study shows that non-branded turmeric powders had less total curcuminoids (11 or 17 mg/g dry weight) than most of the branded ones (≥20 mg/g dry weight). Only one of the branded turmeric powders (BC-6) had low total curcuminoid content (<14 mg/g dry weight) (Table 2).

## Advantages of our method

In comparison to earlier analyses, our method of HPLC analysis yielded lower LOD and LOQ values than the previous ones (see Supplementary Table S1 online). Being highly sensitive, our method can be adapted for qualitative and quantitative analysis of curcuminoids. Besides high accuracy, our method had also excellent recovery of CU (see Supplementary Table S2 online). Possible several reasons for the significant improvement in our analytical procedure are, for e.g. the small particle size (<2 µm) of column and slow flow rate of solvent (Table 3). The current availability of standards of all three curcuminoids of high purity was also helpful for our method. In a study,

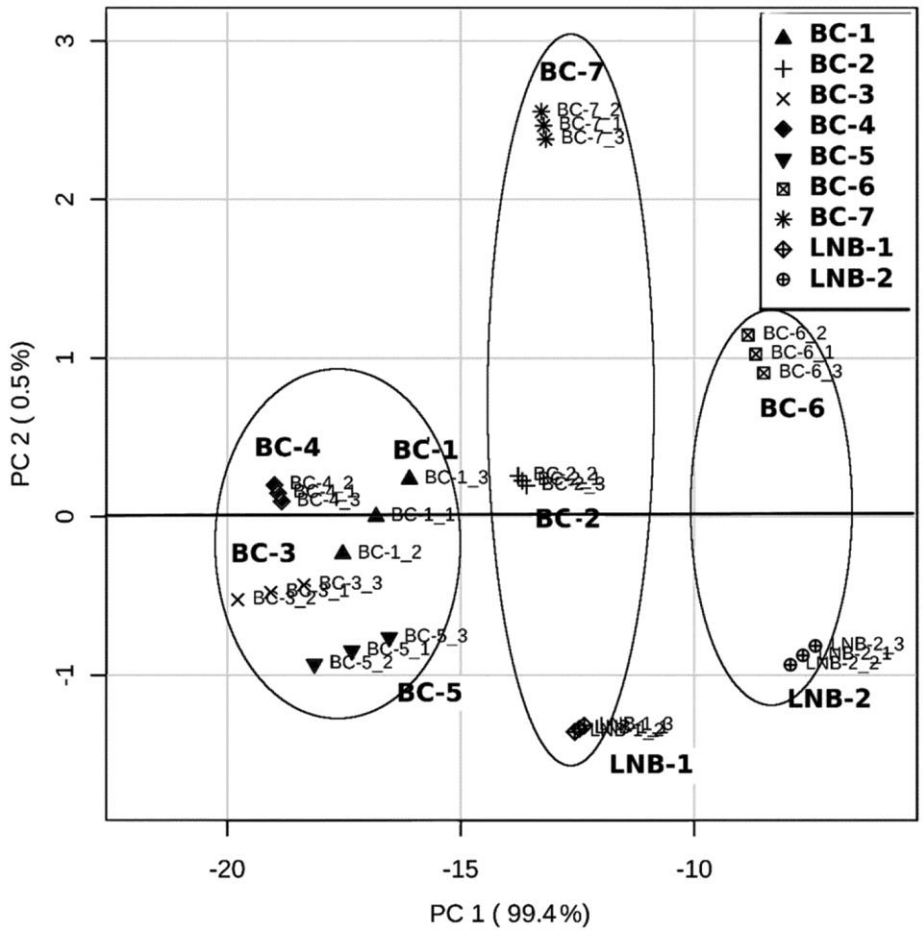


**Figure 4.** Principal component analysis (PCA) plot of the nine commercial turmeric powders based on the amount of three curcuminoids, BDMC, DMC and CU quantitated using HPLC-DAD analysis. Commercial turmeric powders. BC, Branded commercial; LNB, Local non-branded.

standard CU used for HPLC analysis was 80% pure[28]. The availability of pure curcuminoids was limited, some of the researchers had to isolate curcuminoids from turmeric rhizomes[17] or synthesize in their laboratory[18,29] for use as standards. Highly pure commercially available standard curcuminoids are used in the present study for validation of our protocol. We also ensured curcuminoid elution byMS/MS spectra obtained from LC-QTOFMS profile. The two advantages of our methodology are the lowest LOD and LOQ values and the consumption of much lower amounts of solvent than in earlier methodologies[18,21] particularly acetonitrile, disposal of which is a concern for environmental safety. The slow flow rate improved the sensitivity, decreased the volume of solvent used, however, analytical time was about 10 min longer than other protocols. The HPLC method developed and validated by us can be used to quantitate individual curcuminoid content (CU, DMC and BDMC).We have also tested recovery to evaluate the accuracy in quantitation. The recovery of CU in our method by spiking with standard CU was 100% (see Supplementary Table S1 online). Our study would be therefore be useful and appropriate for scientific validation of *Curcuma* rhizomes while assessing their curcuminoid content for commercial turmeric preparation.

## Concluding remarks

Three curcuminoids, namely CU, DMC and BDMC, are major constituents of turmeric rhizomes. Our study provides a quantitative analysis of these three curcuminoids in rhizomes of seven cultivars: five of *Curcuma longa* (cvs. Alleppey Supreme, Duggirala Red, Prathibha, Salem and Suguna) and two of *C. aromatica* (cvs. Kasturi Araku and Kasturi Avidi). The supremacy of two cultivars: Alleppey Supreme and Duggirala Red of *C. longa*, is indicated by high levels of CU along with DMC and BDMC. The high levels of curcuminoids in two cultivars, Kasturi Araku and Kasturi Avidi, suggest that *C. aromatica* can be a potent source of curcuminoids. Besides the curcuminoid levels, their ratios, need to be considered to distinguish the *Curcuma* cultivars. The quality of commercial turmeric powders can also be assessed by determining the curcuminoid profile by HPLC. We

recommend that our HPLC-based method can be an effective tool to differentiate the cultivars of *Curcuma* species and assess the quality of turmeric powders.


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