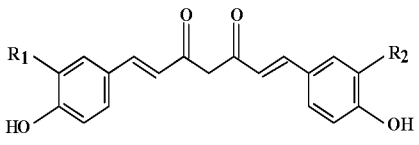
Quantitative determination of curcuminoids in turmeric powder by HPTLC technique

Turmeric (*Curcuma longa* L.), belonging to the family Zingiberaceae, is a rich source of curcuminoids, which are a group of phenolic compounds composed mainly of curcumin (C), demethoxy curcumin (DMC) and bisdemethoxy curcumin (BDMC; Figure 1). They have a wide range of medicinal and culinary uses[1,2]. The content of total curcuminoids in turmeric powder plays an important role in its antioxidant activity and effectiveness of the product[3]. Thus, a sensitive and accurate analytical procedure is required for the study of C, DMC and BDMC in different turmeric samples.

Analysis of botanical compounds can help ensure quality and batch-to-batch reproducibility of botanically derived products. The active chemical constituents in a plant material serve as a characteristic fingerprint for that plant and help develop analytical techniques to ascertain the quantity of the active constituents in botanically derived products. Most of reported methods for the determination of curcuminoids are spectrophotometric in nature, expressing the total colour content of the sample[4,5]. These methods are less sensitive due to the presence of other pigments present in the plant. It is not possible to quantify the individual curcuminoids using the spectrophotometric method. Gupta *et al.*[6] reported the HPTLC method to determine curcuminoids in turmeric. The linearity was found in the concentration range 1–20 μg scanning by UV detection mode. Verma and Joshi[7] developed a rapid HPTLC method for quantification of curcumin in ayurvedic formulation showing a linearity range of 50–250 ng of curcumin. Rasmussen *et al.*[8] reported the separation of curcuminoids using dihydrogen phosphate-impregnated silica gel TLC plates. The analysis of individual curcuminoids by HPLC in normal phase and reverse phase column using different mobile phases has been reported[9–15]. However, the HPTLC method has some unique advantages over HPLC. Sample treatment is simple because of single use of the layer. The time for analysis on a per-sample basis is low, because multiple samples can be run with standards in a single plate using a low volume of solvents, rather than performing sequential injection of samples and standards in HPLC. Simultaneous chromatography of samples and standards under identical conditions on the same layer leads to results with accuracy and precision. Therefore, it was thought worthwhile to develop a simple, rapid and high-precision HPTLC method for determination of individual curcuminoids from commercial turmeric powder.



Curcumin: $R_1 = R_2 = OCH_3$

Demethoxycurcumin: $R_1 = H, R_2 = OCH_3$

Bisdemethoxycurcumin: $R_1, R_2 = H$

Figure 1. Structure of curcuminoids.

The different commercially available turmeric powders (m_1, m_2, m_3, m_4 and m_5) were obtained from a market in West Bengal. Analytical standards of curcuminoids were obtained from Sigma Aldrich, USA. All solvents/chemicals used were of AR-grade and were obtained from E-Merck, Mumbai. The HPTLC pre-coated plates silica gel 60 GF_{254}, 20 × 10 cm, layer thickness 0.2 mm used were from Camag, Muttenz, Switzerland.

Processed turmeric powders (1 g) were ultrasonically extracted with methanol (20 ml × 3) for 15 min and filtered using Whatman No. 42 filter paper. The extract was concentrated under reduced pressure at 50°C in a rotary vacuum evaporator and redissolved in 1 ml of methanol for HPTLC analysis. Extraction of each sample was done in triplicate.

Chromatography was performed on a pre-activated silica gel HPTLC plate ($60GF_{254}$, 20 × 10 cm, 0.2 mm layer thickness). Methanol solution of samples (4 μl) and known concentrations of standards (1–1000 ng) were applied to the plate as 6 mm wide bands using the Camag 100 μl sample syringe (Hamilton, Bonaduz, Switzerland), with an Automated Camag TLC applicator, Linomat 5 with N_2 flow (Camag, Muttenz, Switzerland), @ 150 nl/s, positioned 15 mm from the bottom and 20 mm from side of the plate. The space between two bands was 11 mm. The application parameters were identical for all the analyses performed.

The HPTLC plates were developed in a CAMAG twin trough glass tank (20 × 10 cm) which was pre-saturated with the mobile phase chloroform–methanol (48 : 2, v/v, 20 ml) for 30 min; the length of each run was 7 cm. The TLC runs were performed under laboratory conditions of 25 ± 5°C and 50% relative humidity. The plates were then dried in air. For quantification, the TLC spots corresponding to sample and standard were quantified at 425 nm using a Camag TLC scanner model-3 equipped with Camag Wincats software and a tungsten source, slit width 5 × 0.45 mm, absorption–reflection scan mode and a scanning speed at 5 mm/s. The Wincats software controlling the densitometer produces a calibration plot by linear regression relating standard concentration to the scan area and curcuminoids in samples were automatically interpolated from the calibration curve.

The composition of the mobile phase for TLC was optimized using different solvents (Table 1) of varying polarity and good resolution was achieved using chloroform–methanol (48 : 2) as mobile phase (Figure 2). The R_f values for C, DMC and BDMC were 0.64, 0.38 and 0.22 respectively (Figure 2). The scanning wavelength selected was 425 nm, the absorption maxima of the curcuminoids spot. The peak corresponding to curcuminoids, checked via addition of standard, was well resolved for identification (Figures 2 and 3). Different concentrations of 40, 60, 80 ng of the three curcuminoids were added to the sample solution of the extract (1 g/ml) and analysed by HPTLC. The recovery rates were 98% for C, 96% for DMC and 98% for BDMC respectively.

The average content of C, DMC and BDMC (Table 2) in the different commercial turmeric powders were expressed as gram per 100 g of dry samples. It is clear that curcuminoids were present in each sample having maximum concentration of C followed by DMC and BDMC.

The purity of C, DMC and BDMC spots was confirmed by superimposition of the UV–visible spectra recorded from the plate, using a TLC scanner, from standard and sample zones within the same R_f window (Figure 4).

Detection limit of the three curcuminoids was determined by plotting a series of concentrations on the plate and scanning at 425 nm. The lowest amount of curcuminoid, which could be detected, was 10 ng/spot which was more sensitive than the HPLC method[9]. The lowest amount of curcuminoid which could be quantified was found to be 40 ng/spot.

For determination of the linearity curves of area vs concentration, different amounts (1–1000 ng) of stock solution of C, DMC and BDMC were applied on the HPTLC plate and analysed as above. Calibration was linear in the concentration range 40–1000 ng. The linear regression equation was $Y = 4447.26 + 61.993X$, $Y = 1089.881 + 70.003X$ and $Y = 2611.84 + 51.565X$ for C, DMC and BDMC respectively, while the correlation coefficients (r^2) were 0.9999, 0.9998 and 0.9998 respectively, with high reproducibility and accuracy.

The intra-day reproducibility was evaluated by measuring the sample repeatedly, and inter-day reproducibility was evaluated by analysing the sample for three days. The relative standard deviation precision for intra-day analysis of C, DMC and BDMC ranged from 0.12 to 0.54%, 0.19 to 0.30% and 0.15 to 1.79% respectively. While the inter-day precision of three curcuminoids ranged from 0.64 to 1.36%, 0.35 to 1.5% and 0.42 to 3.75% respectively.

The HPTLC method for the determination of C, DMC and BDMC from turmeric powder reported here is simple, sensitive, economic and accurate, with good precision. Moreover, visual observation and direct recording of the entire chromatogram, including all sample components and the ability to repeat detection and quantification steps under different conditions make the HPTLC method more

Table 1. Different mobile phases for the separation of curcuminoids by TLC

TLC mobile phase (v/v)	R_f value		
	C	DMC	BDMC
Chloroform : benzene : methanol (80 : 15 : 5)	0.61	0.40	0.30
Chloroform : methanol (95 : 5)	0.65	0.43	0.31
Chloroform : methanol (48 : 2)	0.64	0.38	0.22
Benzene : ethyl acetate (9 : 1)	0.75	0.54	0.46

C, Curcumin; DMC, Demethoxycurcumin; BDMC, Bisdemethoxycurcumin.

Table 2. Percentage of curcuminoids content in the extracts of different commercial samples of turmeric powder by HPTLC

Sample	Curcuminoid content in sample extracts (% w/w of samples)*			
	C	DMC	BDMC	Total content of curcuminoids
m_1	0.99 ± 0.13	0.53 ± 0.07	0.49 ± 0.03	2.01 ± 0.23
m_2	0.79 ± 0.07	0.35 ± 0.06	0.34 ± 0.09	1.48 ± 0.22
m_3	0.70 ± 0.12	0.38 ± 0.10	0.30 ± 0.02	1.38 ± 0.24
m_4	0.61 ± 0.06	0.36 ± 0.08	0.26 ± 0.05	1.23 ± 0.19
m_5	0.23 ± 0.02	0.16 ± 0.01	0.13 ± 0.01	0.52 ± 0.04

*Expressed as mean values ($n = 3$) ± SD.

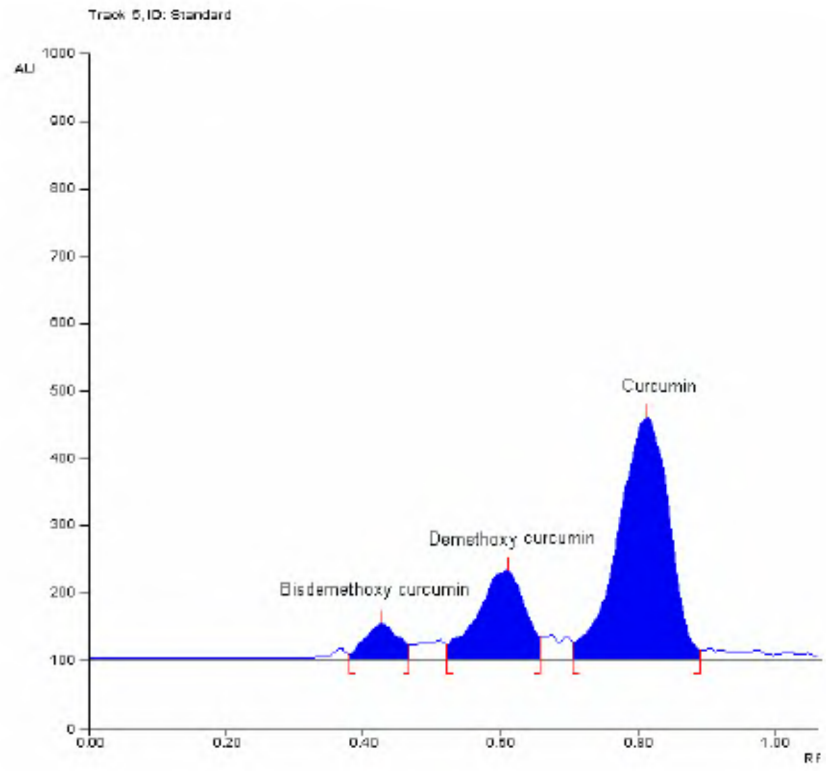


Figure 2. Scan (at 425 nm) of an HPTLC layer showing separation of curcumin, demethoxy curcumin and bisdemethoxy curcumin standard.

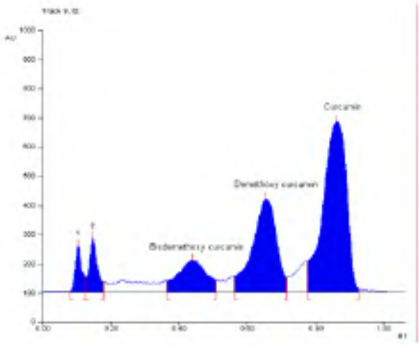


Figure 3. Scan (at 425 nm) of an HPTLC layer showing separation of the three curcuminoids in the extract of turmeric powder (peaks 1 and 2 were not identified in this study).

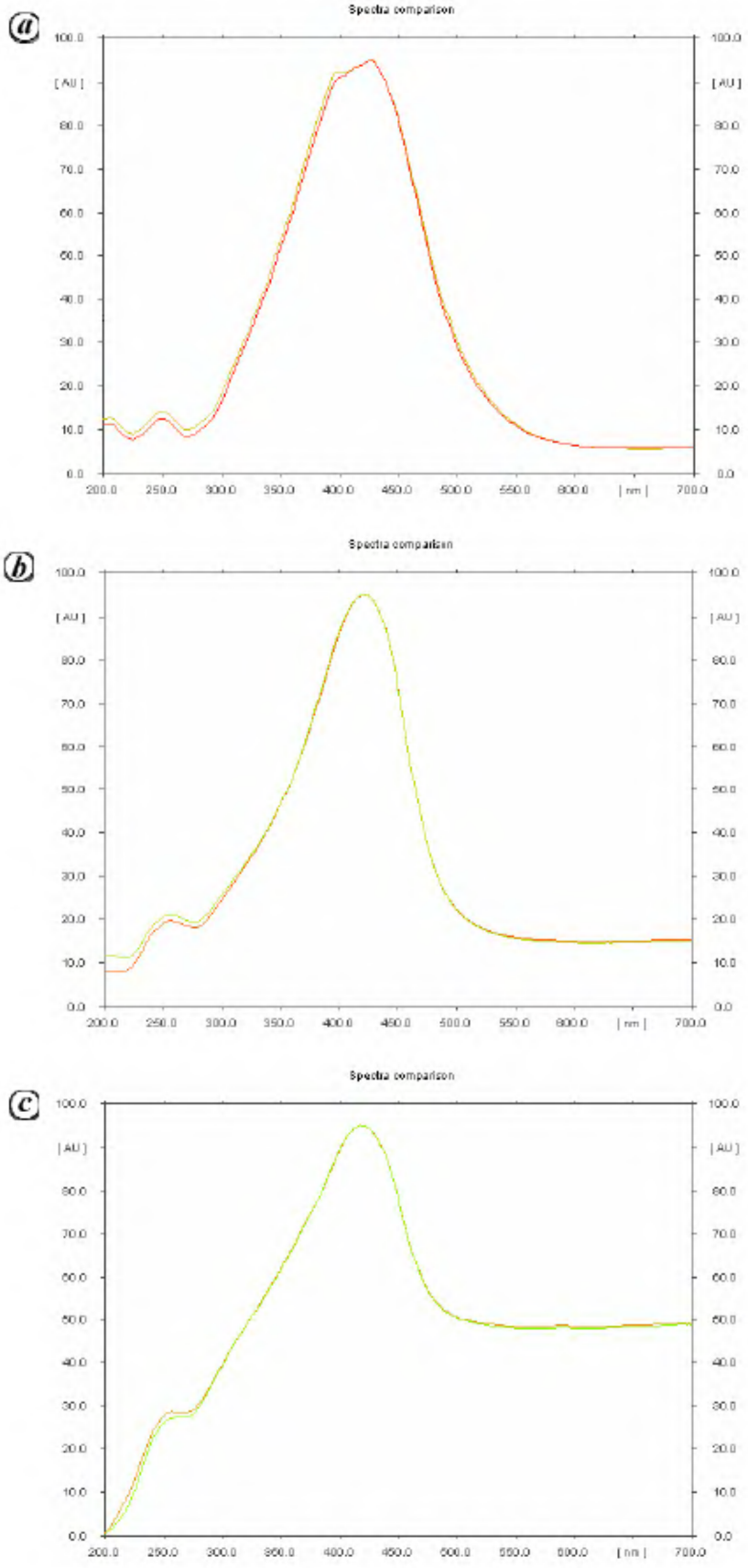


Figure 4. Superimposed spectra of curcumin (***a***), demethoxy curcumin (***b***), and bisdemethoxy curcumin (***c***) from standard and sample zones.

suitable for rapid analysis of a large number of commercial samples of turmeric powder.

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