Wageningen Academic Publishers

UPLC-PDA estimation of curcuminoids as marker compounds for *Curcuma longa* L. in spice mixtures and herbal formulations

A. Ranjith[1*], K.R. Menon[2], S. Roshny[1] and P. Gunjan[1]

[1]Spices Board Quality Evaluation laboratory, Sector-19A, Vashi, Navi Mumbai 400703, India; [2]Spices Board Quality Evaluation Laboratory, Palarivattom, Cochin, Kerala 682025, India; ranjith.arbr@gmail.com

Received: 9 February 2016 / Accepted: 29 July 2016

RESEARCH ARTICLE

Abstract

A simple, sensitive and precise ultra-performance liquid chromatography (UPLC) method for the analysis of curcuminoids in spice products was developed and compared with high-performance liquid chromatography method. The method was validated in terms of linearity, range, precision, accuracy and the results of validation studies were within the acceptable range. The UPLC method was applied to analyse curcuminoids in spice mixtures, herbal pharmaceutical and cosmeceutical formulations to establish curcuminoids as marker compounds for the standardisation of turmeric products. The indicative content of turmeric in the formulations was also calculated by considering 1 g/100 g of curcuminoids in turmeric and most of the formulations were found to contain lesser amount of turmeric than that in label claim.

Keywords: UPLC, *Curcuma longa*, curcumin, curcuminoids

1. Introduction

The bio potency and toxicity of herbal formulations and nutraceuticals largely depend on their phytochemical composition, physical and chemical nature of the product and processing methods. Recent evidences on promising physiological effects of herbal formulations prompted systematic investigation including evidence based validation of health claims and quality and safety assessment of traditional medicinal formulations. The phytochemicals in plants are subjected to wide variations due to varietal differences and geo-climatic conditions. Standardisation in terms of quantitative profiling of active principles from plant to their end products, including their stability, therefore, is vital to provide credence to the health claims and for quality assurance.

Turmeric (*Curcuma longa* L.), a perennial herb belonging to *Zingiberaceae* family, is cultivated extensively in south and southeast tropical Asia. Turmeric rhizome is a major dietary spice and also used to impart colour to food, textile and treat a wide variety of ailments. It is widely used in Asian traditional medicine to cure biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, inflammation and sinusitis (Chattopadhyay *et al.*, 2004). Curcuminoids which constitutes 1 to 10% of the dry rhizome are considered as major bioactive compounds in turmeric. Curcuminoids refer to a group of phenolic compounds in which curcumin (C-1), demethoxycurcumin (C-2) and bisdemethoxycurcumin (C-3) are the major curcuminoids in turmeric (Chattopadhyay *et al.*, 2004; Lekshmi *et al.*, 2014). The potential uses of curcuminoids in medicine have been studied extensively, which includes its anti-inflammatory (Ramsewak *et al.*, 2000), antioxidant (Kim *et al.*, 2005), anticarcinogenic (Anuchapreeda *et al.*, 2008), antidiabetic (Lekshmi *et al.*, 2014; Merrell *et al.*, 2009), antimicrobial (Suvarna *et al.*, 2014), antifibrotic (Punithavathi *et al.*, 2000), hypotensive and hypocholesteremic activities (Lekshmi *et al.*, 2014). Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects (Prashar *et al.*, 2011).

Methods including high-performance thin layer chromatography (Zhang *et al.*, 2008) and high-performance liquid chromatography (HPLC) (Jadhav *et al.*, 2007; Jayaprakasha *et al.*, 2002; Wisut *et al.*, 2009; Zhang *et al.*,

2009) analysis have been reported for the determination of curcuminoids in turmeric. In the present study a sensitive ultra-performance liquid chromatography (UPLC) method was developed, validated and compared with HPLC method. The UPLC method was further extended to quantify curcuminoids as chemical markers in various spice products, herbal cosmeceutical and pharmaceutical formulations.

2. Materials and methods

Materials and reagents

Turmeric rhizome, spice mixtures and herbal formulations were procured from the authorised retail shops in Mumbai, India. Curcumin (C-1) standard was purchased from Sigma Aldrich, St. Louis, MO, USA. HPLC grade methanol, acetone and acetonitrile were purchased from Merck (Mumbai, India).

Preparation of stock and working standard solutions

Hundred mg of C-1 was weighed and dissolved in 100 ml methanol to prepare the stock solution (1000 mg/l). Stock solution was diluted to appropriately with methanol to prepare the working standard solutions of 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/l concentrations.

UPLC analytical conditions

UPLC was performed using a Water Acquity H class system equipped with quaternary solvent delivery pump, a sample manager (FTN) and photodiode array detector (PDA). The separation was carried out using a reversed phase C18 UPLC column (ODS-2, 2.1×100 mm, 1.7 µm; Acquity, Dublin, Ireland). The mobile phase consisting of a mixture of acetonitrile and acidified water (1 ml acetic acid in 100 ml water) in the ratio 50:50 (v/v) with the flow rate of 0.3 ml/min. The detector wavelength was set at 440 nm. Injection volume was 2 µl while the column was maintained at 50 °C.

HPLC analytical conditions

HPLC analysis was performed on a Shimadzu HPLC system containing an LC-20AD liquid chromatography pump unit (Kyoto, Japan),), a Luna®C18 column (250×4.6 mm, 5 µm; Phenomenex, Torrance, CA, USA) with an extended guard column of the same material, a column oven (CTO-20AC; Shimadzu, Kyoto, Japan), an auto sampler (SIL 20A HT; Shimadzu), an auto injector (Rheodyne, Rohnert Park, CA, USA), and a UV/VIS PDA detector (SPD-M20A; Shimadzu). The mobile phase was a gradient elution of acetonitrile and water in the ratio 70:30 (v/v) with the flow rate of 1 ml/min, kept at an oven temperature of 40 °C. A sample of 20 µl was injected onto the column and detection wavelength was 440 nm.

Extraction of curcuminoids from turmeric powder, spices, cosmetics and polyherbal formulations

Powdered turmeric (1 g) or spice mixtures (5 g) was shaken with 100 ml acetone at 180 rpm for 30 min and filtered through a Whatman filter paper (11 µm particle retention; GE Healthcare UK Limited, Chalfont St Giles, UK).1 ml of the extract was diluted to 100 ml with methanol and subjected to UPLC or HPLC analysis. Herbal products (1 g) except Dasamularishtam was extracted with 100 ml acetone by shaking at 180 rpm for 30 min, filtered and injected to UPLC. Extracts were filtered through 0.4 µ syringe filters and injected to UPLC or HPLC for the analysis. The peaks obtained by running the samples in HPLC or UPLC were compared with those obtained for C-1 and the content of curcuminoids was estimated in terms of C-1.

Method validation

The developed HPLC and UPLC methods were validated in terms of its selectivity, peak purity, linearity, limits of detection, limits of quantification, recovery precision and robustness.

3. Results and discussion

UPLC-PDA analytical method for curcuminoids

Varying compositions of acetonitrile-water mixtures were tried as mobile phase for the separation of curcuminoids. Oven temperature was also found to have a major role in the resolution of curcuminoids in UPLC column of 1.7 µm particle size. Results of the optimisation trials are summarised in Table 1. An isocratic mobile phase of

Table 1. Effect of mobile phase combination and oven temperature on the ultra-performance liquid chromatography separation of curcuminoids.

Acetonitrile	Water	Oven temperature (°C)	Resolution of peaks
70	30	35	poor separation
60	40	35	poor separation
55	45	35	poor separation
50	50	35	separation with peak tailing
Acidified mobile phase (acetonitrile with 1% acetic acid)			
60	40	40	poor separation
		50	poor separation
55	45	40	poor separation
		50	poor separation
50	50	40	poor peak shape
		50	good separation

acidified acetonitrile-water mixture at column temperature 50 °C separated C-1, C-2 and C-3 into peaks of good shape within 5 min of run time. UPLC method was able to detect curcuminoids in shorter time with more sensitivity than HPLC method (Table 2, Figure 1).

Selectivity of the method was established by overlapping UV-visible spectra at start, middle and end points of each peak. An overlapping symmetry of >90% was used as the criteria for selectivity. The UPLC and HPLC methods were validated in terms of linearity range, precision, accuracy, robustness and the results are given in Table 2. Since the availability of C-2 and C-3 in pure reference grade is limited, linearity and recovery studies were performed with C-1 only. C-2 and C-3 share a common diphenylheptanoid skeleton and differs only in number of methoxy groups and not much variation is shown in absorption spectrum. C-1 showed a linear response in UPLC with regression equation y = 36,435x – 59 within the range of 0.1 to 2 ng. The linearity range for the HPLC method was 0.5-10 ng. Repeatability study results of C-1, C-2 and C-3 were within the acceptable limits. The method was able to recover 91.6% C-1 from the fortified turmeric powders. The method was robust against deviations up to 3% in mobile phase composition, flow rate and column oven temperature. HPLC method was more robust by allowing deviations up to 5% in these parameters.

Five turmeric samples were analysed simultaneously in UPLC and HPLC analytical methods and compared each other to establish the reliability of UPLC method. The analysed turmeric samples contained 1.02 to 4.93 g/100 g of total curcuminoids. Each sample was analysed 5 times in UPLC and HPLC methods and mean values were compared by matched pair student's T-test. The observed T (T_{obs}) was calculated using the equation:

$$T_{obs} = \bar{d}i / \sqrt{[(\Sigma di^2 - \frac{1}{n}(\Sigma di)^2)/n(n-1)]}$$

Where, T_{obs} is the observed T value; d is the deviation between the values; and n the sample size. T_{obs} obtained for curcuminoid in all the trials was lesser than the critical T value (2.78) in the Students distribution table for a risk factor of 5%. This indicated that means of differences between UPLC and HPLC analytical results were not significant.

Analysis of turmeric powder and spice mixtures

Twenty numbers of dried turmeric rhizome obtained from various sources were analysed in UPLC. Total curcuminoid content in these samples varied from 0.72 to 6.23 g/100 g with an average C-1:C-2:C-3 ratio of 4.5:1.5:1 (data not shown). This ratio might be considered as natural proportion of curcuminoids and could be used to distinguish between natural and synthetic curcuminoids. Jayaprakash *et al.* (2002) estimated the curcuminoid content in Indian varieties of turmeric using HPLC and reported 2.34 to 9.18 g/100 g of total curcuminoids. Total curcuminoid content of 0.66 to 11.05 g/100 g was reported in turmeric powders collected from markets in China (Zhang *et al.*, 2009). All these reports indicate C-1 as the major curcuminoid followed by C-2 and C-3. The content of curcuminoids in spice mixtures are given in Table 3. Spice mixtures belonging to different classes of curry powders and one instant mix were analysed for curcuminoid content by assuming a content 1 g/100 g of curcuminoids in turmeric powders. The content of turmeric powder in the mixture was also calculated and compared with that of label claim. It was noted that most of the spice mixtures contained lesser turmeric powder than in the label claim. The proportion of individual compounds in the mixtures was in accordance with that of turmeric.

Table 2. Validation data of ultra-performance liquid chromatography (UPLC) and high-performance liquid chromatography (HPLC) methods (values are average or average ± standard deviation, n=5).[1]

Parameters		HPLC			UPLC		
		C-1	C-2	C-3	C-1	C-2	C-3
Linearity[2]		y = 128,318x – 4,261			y = 36,435x – 59		
Range (ng)[2]		5-100			0.5-10.0		
LOQ (ng)[3]		0.5	0.5	0.5	0.1	0.1	0.1
Repeatability (% RSD)	T_R	0.27	0.26	0.24	0.33	0.29	0.28
	Area	1.53	1.14	1.59	1.90	1.78	185
% Recovery[2]		94.2±1.9			91.6±2.6		

[1] C-1 = curcumin; C-2 = demethoxycurcumin; C-3 = bisdemethoxycurcumin.
[2] Studies performed with C-1 only.
[3] LOQ = limits of quantification.

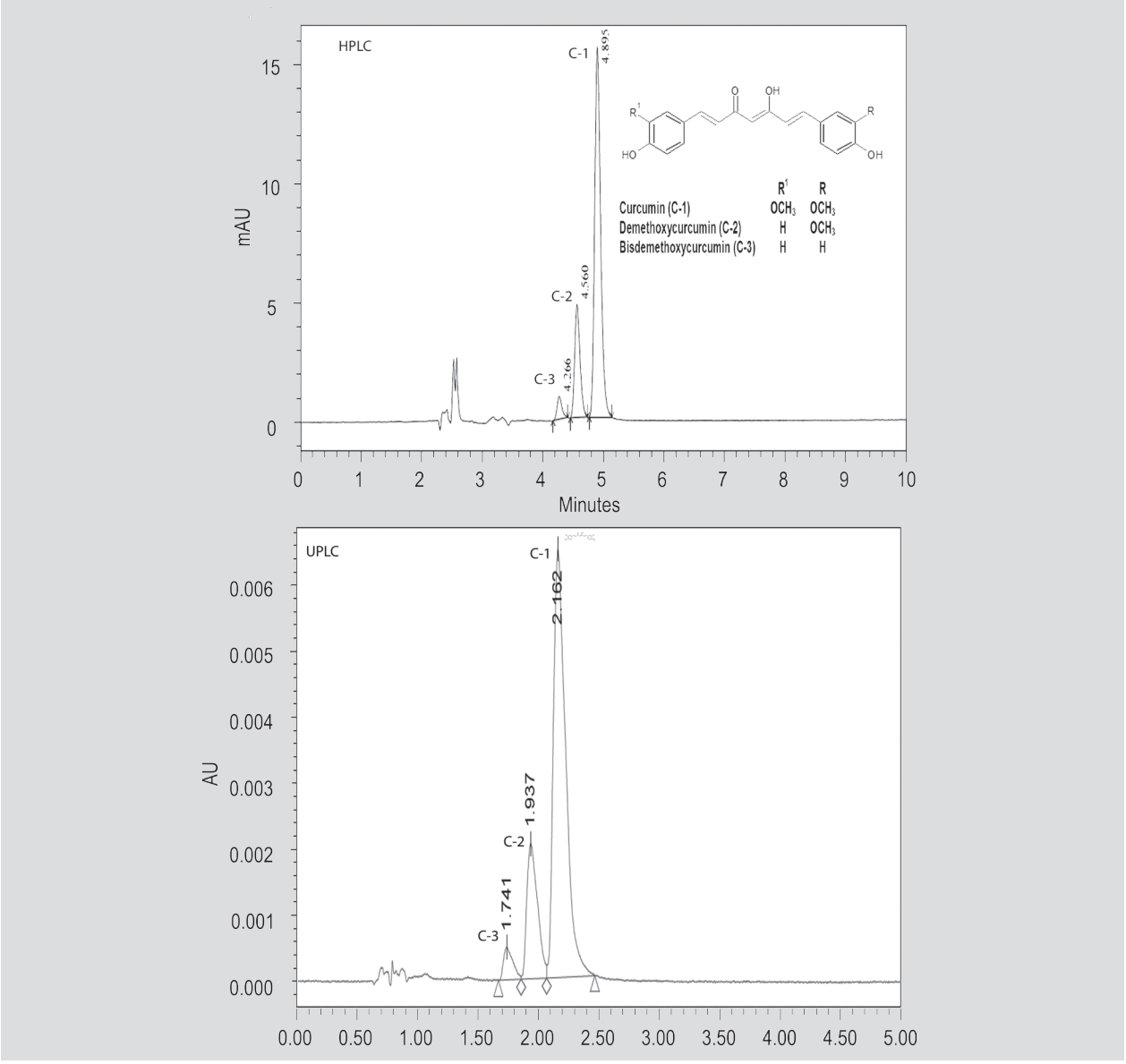

Figure 1. Ultra-performance liquid chromatography (UPLC) and high-performance liquid chromatography (HPLC) chromatogram of curcuminoids in turmeric powder.

Analysis of herbal preparations

Herbal cosmetic and medicinal formulations were analysed in UPLC for their curcuminoid content and indicative turmeric content was also calculated by considering 1 g/100 g curcuminoid content in dry turmeric rhizome. (Table 3). Herbal cosmetic preparations belonging to the classes of face cream, face pack, foot cream, body lotion, etc. of reputed manufacturers were analysed for curcuminoid contents. Many of the products were not detected for curcuminoids in contrary to their label claims. The products which detected for curcuminoids contained lesser amount of turmeric than the label claims. Herbal pharmaceutical formulations of different forms such as water decoction (Dasamoolarishtam) tablets, granules and powders were also analysed. All the analysed products contained curcuminoids in a lesser quantity than the label claim. The herbal cosmetic and medicinal formulations containing considerable amount of curcuminoids seemed to have natural proportion of C-1, C-2 and C-3.

The variation in natural content of curcuminoid in turmeric and thermolabile and photosensitive nature of curcuminoids limit the possibility to link curcumin content directly to turmeric content in the formulations. However, the indicative nature of curcuminoids could be utilised to standardise herbal formulations. A scale of curcumin concentration in the products corresponding to the turmeric content by considering the natural variations and processing loss could be formulated to standardise

Table 3. Ultra-performance liquid chromatography analysis of spices mixtures and herbal cosmeceutical and medicinal formulations.[1,2]

Product name		Curcuminoids content (mg/kg)[3]				% of turmeric[4]	
		C-1	C-2	C-3	Total	Label	Analysis
Spice mixtures							
Sambar powder	1	462	164	118	744	NS	7.44
	2	330	98	66	494	NS	4.94
	3	124	38	28	190	NS	1.90
Meat masala	1	704	236	178	1,118	NS	11.18
	2	463	147	107	717	NS	71.70
	3	976	298	196	1,470	NS	14.70
Egg curry masala		2,124	620	416	3,160	NS	31.60
Chicken masala		1,914	658	502	3,074	NS	30.74
Vegetable masala		2,116	644	428	3,188	NS	31.88
Pickle masala		834	260	138	1,232	NS	12.32
Pavbhaji masala		520	240	180	940	NS	9.40
Noodles masala		548	196	112	856	NS	8.56
Instant vegetable soup		8	6	2	16	NS	0.16
Herbal cosmetic formulations							
Skin cream		66.3	31.7	27.4	125.4	16.0	0.8
Face wash	1	ND	ND	ND	ND	0.5	127.4
	2	ND	ND	ND	ND	0.5	914.9
	3	ND	ND	ND	ND	NS	823.2
Face pack		2.0	ND	ND	2.0	NS	0.02
Face cream		1.1	ND	ND	ND	NS	0.01
Foot cream		ND	ND	ND	ND	1.10	ND
Body lotion		ND	ND	ND	ND	0.02	ND
Oral gel		ND	ND	ND	ND	0.01	ND
Herbal medicinal formulations							
Dasamularishtam		0.8	ND	ND	0.8	0.24	0.00
Tablet (detoxifier)		95.5	19.9	12.0	127.4	0.07	12.74
Anti-diabetic granule		583.9	189.4	141.6	914.9	16.70	9.15
Herbal powder		554.5	169.9	98.8	823.2	9.60	8.23

[1] Values are average ± standard deviation, n=5.

[2] C-1 = curcumin; C-2 = demethoxycurcumin; C-3 = bisdemethoxycurcumin; ND = not detected (<0.1 ng); NS = not specified.

[3] Values reported in terms of curcumin.

[4] % of turmeric content calculated by assuming 1 g/100 g total curcuminoid content in turmeric powder.

the formulations. This would offer a way to confirm the turmeric content claimed in the label of products. The proportion of individual curcuminoids in the products could be utilised to ensure the natural origin of curcuminoids.

References

Anuchapreeda, S., Tima, S., Duangrat, C. and Limtrakul, P., 2008. Effect of pure curcumin, demethoxycurcumin, and bis-demethoxycurcumin on WT1 gene expression in leukemic cell lines. Cancer Chemotherapy and Pharmacology 62: 585-594.

Chattopadhyay, I., Biswas, K., Bandyopadhyay, U. and Banerjee, R.K., 2004. Turmeric and curcumin: biological actions and medicinal applications. Current Science 87: 44-53.

Jadhav, B.K., Mahadik, K.R. and Paradkar, A.R., 2007. Development and validation of improved reversed phase-HPLC method for simultaneous determination of curcumin, demethoxycurcumin, and bis-demethoxycurcumin. Chromatographia 65: 483-488.

Jayaprakasha, G.K., Rao, L.J.M. and Sakariah, K.K., 2002. Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Journal of Agriculture and Food Chemistry 50: 3668-3672.

Kim, H., Park, B.S., Lee, K.G., Choi, C.Y., Jhang, S.S., Kim, Y.H. and Lee, S.E., 2005. Effects of naturally occurring compounds on fibril formation and oxidative stress of beta-amyloid. Journal of Agriculture and Food Chemistry 53: 8537-8541.

Lekshmi, P.C., Arimboor, R., Nisha, V.M., Menon, A.N. and Reghu, K.G., 2014. *In vitro* antidiabetic and inhibitory potential of turmeric (*Curcuma longa* L) rhizome against cellular and LDL oxidation and angiotensin converting enzyme. Journal of Food Science and Technology 57: 3910-3917.

Merrell, J.G., McLaughlin, S.W., Tie, L., Laurencin, C.T., Chen, A.F. and Nair, L.S., 2009. Curcumin-loaded poly (e-caprolactone) nanofibres: diabetic wound dressing with antioxidant and anti-inflammatory properties. Clinical Experimental Pharmacology and Physiology 36: 1149-1156.

Prashar, D., Khokra, S.L., Purohit, R. and Sharma, S., 2011. Curcumin: a potential bioactive agent. Research Journal of Pharmaceutical Biological and Chemical Sciences 2: 44.

Punithavathi, D., Venkatesan, N. and Babu, M., 2000. Curcumin inhibition of bleomycin induced pulmonary fibrosis in rats. British Journal of Pharmacology 131: 169-172.

Ramsewak, R.S., Dewitt, D.L. and Nair, M.G., 2000. Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins I-III from *Curcuma longa*. Phytomedicine 7: 303-308.

Suvarna, R., Bhat, S.S. and Hegde, K.S., 2014. Antibacterial activity of turmeric against *Enterococcus faecalis* – an *in vitro* study. International Journal of Current Microbiology Applied Sciences 3: 498-504.

Wisut, W., Nutthapon, J., Sunibhond, P. and Pornchai, R., 2009. A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. Phytochemical Analysis 20: 314-319.

Zhang, J., Jinnai, S., Ikeda, R., Wada, M., Hayashida, S. and Nakashima, K., 2009. A simple HPLC-fluorescence method for quantitation of curcuminoids and its application of turmeric products. Analytical Sciences 25: 385-388.

Zhang, J.S., Guan, J., Yang, F.Q., Liu, H.G., Cheng, X.J. and Li, S.P., 2008. Qualitative and quantitative analysis of four species of *Curcuma* rhizomes using twice development thin layer chromatography. Journal of Pharmaceutical and Biomedical Analysis 48: 1024-1028.