Oriental Journal of Chemistry Vol. **25**(1), 105-110 (2009)

Reverse phase high performance liquid chromatographic method for the estimation of *curcumin*

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(Received: June 08, 2008; Accepted: July 10, 2008)

ABSTRACT

A simple, precise, rapid and accurate, binary reverse phase high performance liquid chromatographic method has been developed for the determination of curcumin, Demethoxy curcumin and Bismethoxy curcumin with short run time. Chromatographic separation was achieved by using Merck C_{15-} (250 cm × 4.6 mm) Column with mobile phase tetrahydrofuran: 1% citric acid 35: 65 was used. The flow rate was 1.2 ml/min. The retention time for curcumin was 15.892 minutes. The proposed method was validated for sensitivity, selectivity, linearity, accuracy, precision, ruggedness, robustness and solution stability. The limit of detection and quantification of curcumin was between 1 to 500 ng/mL respectively for 50-µL injection volumes. Limit of detection and Limit of quantification was calculated by visualization and statistical methods. The % recovery of sample spiked standard ranged within 95-105 %. Method, system, interday and intraday precision was found to be within the limits of acceptance criteria. Method was found to be rugged when different analyst carried out analysis. The method was found to be sensitive and efficient with 4886.77 theoretical plates and 0.2046 mm HETP. The method was suitable for the quality control of curcumin therefore can be applied to both *in vitro* studies of curcumin formulations as well as drug estimation in biological samples.

Key words: *Curcumin*, HPLC, natural drug, limit of detection.

INTRODUCTION

Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3, 5-dione] is the major yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb *Curcuma longa* Linn[1]. It is a naturallyoccurring polyphenolic phytochemical currently being examined in preclinical trials for cancer chemoprotective drug development, with pharmacological actions that including antioxidant[1,2], anti-inflammatory[3,4], and cancer chemopreventive actions[5-7]. Curcumin, the major yellow-orange pigment extracted from turmeric, may be responsible for much of the bioactive effects. In a recent study, products of curcumin reduction and conjugation had a reduced ability to inhibit cyclooxygenase-2 (COX-2) expression, which correlated to a decrease in the inhibition of prostaglandin biosynthesis when compared to intact curcumin, indicating that the metabolic conversion of curcumin results in pharmacologic deactivation[8]. Curcumin is also a potent scavenger of various reactive oxygen species (ROS) including superoxide anions[2] and hydroxyl radicals[2,9]. In addition, there have been indications that curcumin may help prevent and treat patients with Alzheimer's disease by reducing oxidative damage, plaque burden, and suppressing specific inflammatory factors[10].

A limitation to the studies cited above was the inability to quantitate low curcumin concentrations and derivatives. Quantitation of curcumin concentrations below 10 ng/ml would allow better characterization and understanding of the disposition and absorption kinetics of this

compound. Although several methods of detection for curcumin have been published, only one has reported a limit of quantitation below 10 ng/ml[8]. Of these methods, several involve spectrophotometric[12], liquid chromatography-mass spectrophotometric[13,14], and radiolabeled determination of curcumin[15]. HPLC methods have also been developed in order to quantitate curcumin in biological samples[8,11,16,17]. Ireson *et al.*[8] utilized a HPLC gradient system that produced reasonable separation and sensitivity. The retention time for curcumin, however, was greater than 35 min. We therefore focused on developing a rapid and more sensitive HPLC binary method for the estimation of Curcumin.

EXPERIMENTAL

Chemicals and reagents

Curcumin was obtained from Natural remedies manufacturing company Bangalore. Tetrahydrofuran and citric acid was obtained from Thomas Baker, India. Water was deionised by the Milli-Q Plus system (Millipore).

Instrumentation

The HPLC system consists of a Shimadzu SPD-10TVP, Binary pump equipped with a normal sample injector with a 50-microliter loop, SPD-10AVP variable wavelength UV detector and Spincotech station for data analysis.

Sample preparation

The stock solutions were prepared by dissolving 5.0 mg of Curcumin was dissolved in 50 ml mobile phase to get a concentration of 1, 00,000 ng/ml. Analytical standard solutions for linearity were prepared by diluting the stock solution with tetrahydrofuran and 1% citric acid immediately prior to use. All the preparations were made in borosilicate glass tubes. The standard calibration curve was constructed in the concentration range of 1-100 ng/ml, with concentration on X- axis, peak area on Y-axis and regression equation was calculated.

Chromatographic conditions

Chromatographic separations were achieved using a Shimadzu ODS $C_{15,--}$ 1 cm long Guard column (4.6×250 mm, 5 µm). The mobile phase consisting of tetrahydrofuran: 1% citric acid 35:65 v/v was passed through a 0.22 mm membrane filter and degassed by ultrasonication under vacuum before use. The flow rate was maintained at 1.2 ml/min and the effluent was monitored for UV absorption at 425 nm. The injection volume was 50 µL. All separations were performed at ambient temperature.

RESULTS

Method development

The objective of this study to develop method for the determination of Curcumin with short run time, which can also be used for its formulations. The column chosen for this study was 250 mm length, 4.6mm internal diameter and 5-micron particle size. Good sample separation was observed on silica based C_{15} Mark column using mobile phase tetrahydrofuran: 1% citric acid 35:65 v/v. The retention time of Curcumin was found to be 15.892 min. The system suitability results were given in Table 1.

Method validation

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method LOD is determined as the lowest amount to detect and LOQ is the lowest amount to quantify by the detector. For statistical method LOD and LOQ determined by statistical formula.

$$LOD = 3.3\ SD/S$$
$$LOD = 10\ SD/S$$

where, SD is standard deviation of y intercept of regression equation and S is slope.

The values for the Limit of Detection and Limit of Quantification for are mentioned in Table 2.

Precision

Precision of the procedure was determined by repeatability method. A solution of Curcumin containing 10ng and 5000ng/ml respectively was injected into the system repeatedly six times. The percentage RSD of injection repeatability and analysis repeatability for Curcumin was found to be

Table 1: Validation data of Curcumin

S. No.	Parameters		Observations	Acceptance Criteria
1.	LOD (mcg/ml)	Visualization	0.2	-
		Statistical	1.33	-
2	LOQ (mcg/ml)	Visualization	0.4	-
		Statistical	4.03	-
3	Linearity	Range (mcg/ml)	1 - 40	-
		Regression eq^n	178913x - 37010	-
		R^2	0.9997	-
4	Accuracy(%Recovery)	Level I (80%)	97.32	% Recovery within
		Level II (100%)	104.50	90 to 120%.
		Level III (120%)	95.56	
5	Precision(%RSD)	Method	0.4324	% RSD should
		System	0.797	be less than
		Interday	0.905	2%.
		Intraday	1.2917	
6	Ruggedness	Analyst 1	99.51	% Assay should be
	(%Assay)	Analyst 2	99.34	within 95-102%.
7	Robustness	% Assay		
	Flow rate (ml/min)	0.8	95.53	should be
	Tetrahydrofuran:	1.2	99.76	within 95-
	1% citric acid	35:65	101.48	102%.
	Wavelength	40:60	98.54	
		425	95.02	

Table 1: System-suitability report

Compound (n=3)	Asymmetry/ Tailing factor	Capacity factor	Efficiency (N) (No. of theoretical plates)	Eff/l [t.p/m] (Relative efficiency in plates per meter)
Curcumin	1.023	5.92	4886.77	37538

n = Number of determinations

Table 2: Recovery results of Curcumin in sample

Added (ng)(n=3)	Recovered	%Recovery	% RSD (n=6)
200 ng	197.49 ng	98.74	1.274
500 ng	497.92 ng	99.58	1.316

n = Number of determinations

0.812 %and 0. 856% % respectively. The results obtained confirm good precision of the method developed.

Linearity

The linearity of the method for Curcumin was checked at ten concentration levels over the concentration range of 50-5000 ng/ml. The typical equation describing the calibration curve is y=0.830x where y is the peak area of Curcumin and x is the concentration of Curcumin, with a mean correlation coefficient (R^2) of 0.9997. Linearity is presented in Fig. 2.

Quantification of Curcumin in natural samples

The standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of Curcumin samples.

The recovery of Curcumin was calculated from the slope and the intercept of the calibration curve drawn in the concentration range of 50-100000ng/ml. The percentage recovery of Curcumin was ranged from 98.6 % to 99.4 % in samples of Curcumin. The results were shown in Table 2. HPLC chromatograph of Curcumin in samples was shown in Figure 1.

Limit of detection

The limit of detection represents the concentration of analyte that would yield a signal to noise ratio equal to 3s(DL=3σ/S)[5]. The limit of detection for Curcumin was found to be 3.68 to 8.125 ng/ml for 50µL injection Volume. The limit of quantification represents the concentration of analyte that would yield a signal to noise ratio equal to 10σ ((DQ=10σ/S)[5]. Limit of quantification for Curcumin was 8.125 ng/ml for 50µL injection Volume.

Solution stability

Solution stability of Curcumin was studied

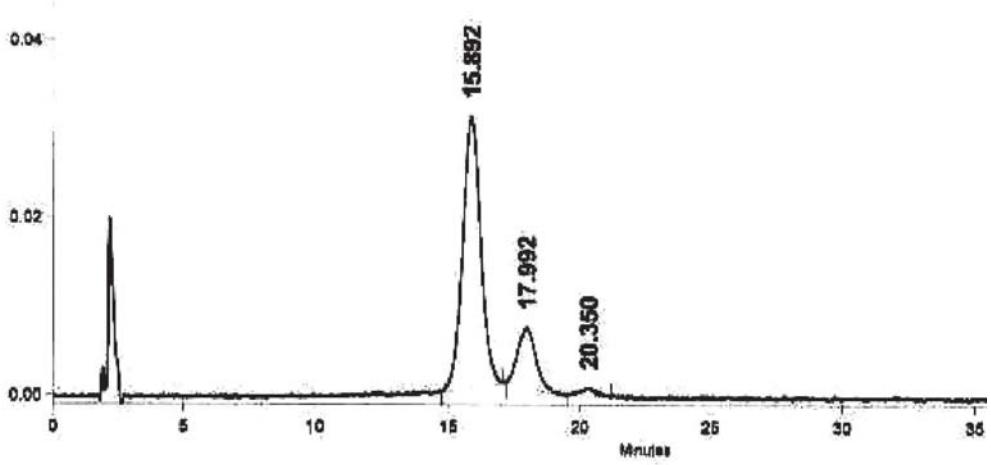

Fig. 1: Chromatograph of Curcumin

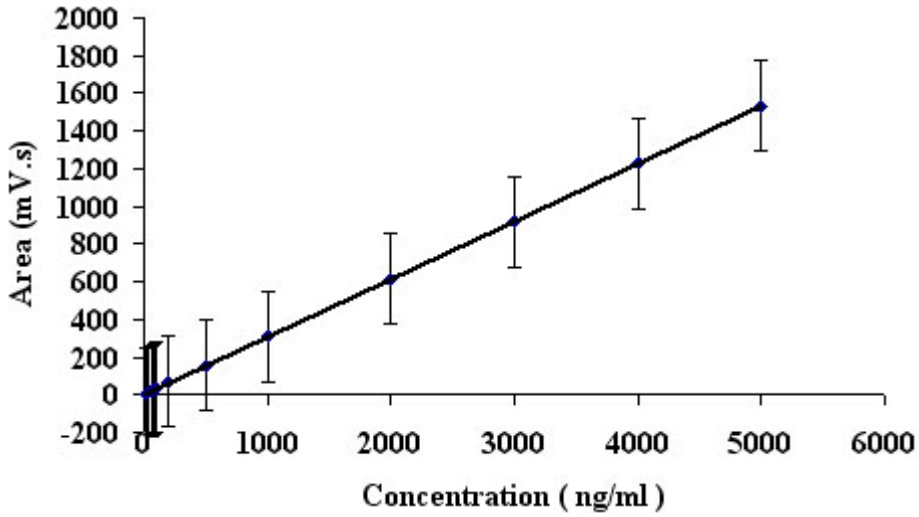

Fig. 2: Linearity graph of curcumin

by leaving the solution (10 and 5000ng/ml prepared in diluent) in tightly capped ambered colour volumetric flasks at room temperature for three days. Content of Curcumin was checked for 12 hours interval and compared with freshly prepared solutions. No variation was observed in the content of Curcumin for the study period, which indicates that the Curcumin sample solutions prepared in the said diluents are stable for at least 3 days.

Ruggedness

The ruggedness was established by carrying out the assay of curcumin using the same chromatographic system and the same column by two analysts on a different day. The assay results were found within the acceptance criteria of 95 to 102% w/w, hence the proposed method was said to be rugged.

Robustness

It is the measure of capacity of an assay to remain unaffected by small but deliberate variations in method parameters and provide an indication of its reliability in normal usage. For the robustness study small variations in columns, mobile phase, detection wavelength and flow rate have been performed and percentage assay of curcumin was calculated. The percentage assay in all varied chromatographic conditions was found within the acceptance criteria.

DISCUSSION

A simple and sensitive HPLC method was developed for Curcumin. This assay method provided excellent sensitivity, accuracy and precision, with relatively short retention time for Curcumin. This HPLC method can therefore be applied to both *in vitro* studies of Curcumin formulations as well as drug estimation in biological samples.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. B.G. Shivananda, Principal, Al-Ameen College of Pharmacy for his kind support and encouragement. This study was supported by SRF grant to Vivek Yadav from Indian Council of Medical Research, India.

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