

Determination Of Curcumin In Capsule Containing *Curcuma xanthorrhiza* Roxb. Extracts By High Performance Liquid Chromatography

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ABSTRACT

Curcumin, the major yellow pigment of *Curcuma sp.* has several interesting biological activities, such as anticarcinogenicity, antiinflammatory, and antioxidant. Curcumin naturally occurs as major compound in Curcuminoid substance beside its derivatives, i.e. Bisdemethoxycurcumin (BDC) and Demethoxycurcumin (DC). Quantitative analysis of Curcumin is difficult to carry out unless prior to completely resolved Curcumin from BDC and DC. A good resolution, reproducible, and accurate method for determination Curcumin in pharmaceutical product (capsule formed) by HPLC has been carry out. Using C₁₈ columnn and mixture of Acetonitrile: Acetic Acid glacial 1% (45:55 v/v) as eluent, BDC, DC, and Curcumin were retained with retention time of 10.79, 12.29, and 13.91 minutes, respectively. Curcumin was simultaneously separated from BDC or DC. The resolution factor (Rs) between Curcumin and DC was 1.73. Good linear relationship ($r = 0.9997$, $V_{xo} = 0.99\%$) between peak area and concentrations was found at range of the Curcumin concentration used, i.e. 23.56-62.83 µg/mL. Curcumin recoveries were (93.60-118.17)% with the coefficient of variation (CV) 6.99%. Curcumin content in each capsule sample was found to be (8.89±0.06) mg. It showed that samples contained higher amount of Curcumin than that claimed on its label, 5 mg.

Keywords: Bisdemethoxycurcumin, Demethoxycurcumin, Curcumin, Curcuminoid, HPLC.

INTRODUCTION

Curcumin [7-Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-dien-3,5-dion] (Fig. 1) is a useful substance for human health. Curcumin naturally occurs as major compound in Curcuminoid substance besides its derivatives, i.e. Bisdemethoxycurcumin (BDC) and Demethoxycurcumin (DC). Curcumin have several interesting biological activities, such as anticarcinogenicity, antiinflammatory, and antioxidant (Verghese, 1999; Majeed, *et al.*, 2000; Stankovic, 2004). Curcumin content in registered pharmaceutical product

must be controlled in order to guarantee whether its concentration conforms to label's declared. Curcumin as a natural product is difficult to be measured quantitatively unless completely resolved from BDC and DC.

The aim of this study was to develop a HPLC method for determination of Curcumin in a registered pharmaceutical product (capsule formed).

MATERIALS AND METHOD

Materials

Curcumin (Sigma-Aldrich, which has 77.1% purity by HPLC) was used as

received from its manufacturer for preparing standard solution in methanol. Curcumin's Certificate of Analysis declared that Standard Curcumin was extracted from *Curcuma longa* (Turmeric). Standard Curcumin solutions were prepared daily (*rp*).

Chemicals

Methanol and Acetonitrile (J.T. Baker) were HPLC grade. Acetic acid (Merck) was pro analysis grade.

Instrument

HPLC Agilent 1100 series equipped with Lichrospher RP 18 column and Diode Array Detector.

Sample

Temulawak capsule which has registration number DEPKES RI No. TR: 963385001. The declared content of each capsule was *Curcuma xanthorrhizae* Roxb. Extract equivalent to 5 mg Curcumin.

Sample Preparation

Ten capsules were opened in order to determine the average weight of their powder content. The powder obtained was homogenized. Powder (*ca.* 265 mg) was weighted accurately and transferred into a 50 mL volumetric flask. Approximately 20 mL of methanol was added and the mixture was placed in vortex agitator for 3 minutes. Furthermore, the flask was filled with methanol up to 50.0 mL and shaken homogenously. A mixture obtained was filtered through a Whatman No. 40 filter paper. The first milliliter filtrate was discarded. A suitable amount of filtrate was diluted quantitatively twice or four times with methanol before it was filtered through 0,2 μm Milipore filters. Twenty microliter

of diluted filtrate was ready to be injected into HPLC system.

Validation of the Method

Optimum condition of HPLC system for separation Curcumin from BDC and DC was obtained by modifying various eluent mixture and eluent flow rate. Identification of Curcumin peak in sample was based on similarity of retention time (t_R), peak's profile, and overlapping of peak's spectra (using match factor value) compared to standard Curcumin. Selectivity parameter was Resolution factor (R_s) between Curcumin and other substances in sample.

The accuracy of the method was validated by spiking samples with standard Curcumin at three fortification level (50-150)% of expected value. Each concentration level was analyzed triplicate (Cunnif, 1995; USP XXVI, 2003). The precision was evaluated by analyzing nine values of percentage recovery obtained in determination of accuracy.

The linearity over the range investigated can be assessed from graph of Standard Curcumin concentration versus area obtained (five times replications).

RESULT AND DISCUSSION

Separation of Curcumin from Its Derivatives

Figure 2 shows the absorption spectra of 1 and 2.5 $\mu\text{g/mL}$ standard Curcumin solution. The ϵ_{max} of Curcumin(420 nm) was then selected for quantitative analysis.

Optimum HPLC system for the separation of Curcumin from other substances was obtained by eluted sample through a mixture of Acetonitrile: Acetic

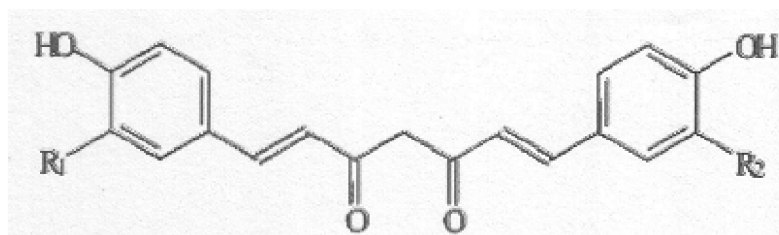


Figure 1. Structure of Curcuminoid

Curcumin	R ₁ and R ₂ = -OCH ₃
Demethoxycurcumin (DC)	R ₁ = -OCH ₃ , R ₂ = -H
Bisdemethoxycurcumin (BDC)	R ₁ and R ₂ = -H

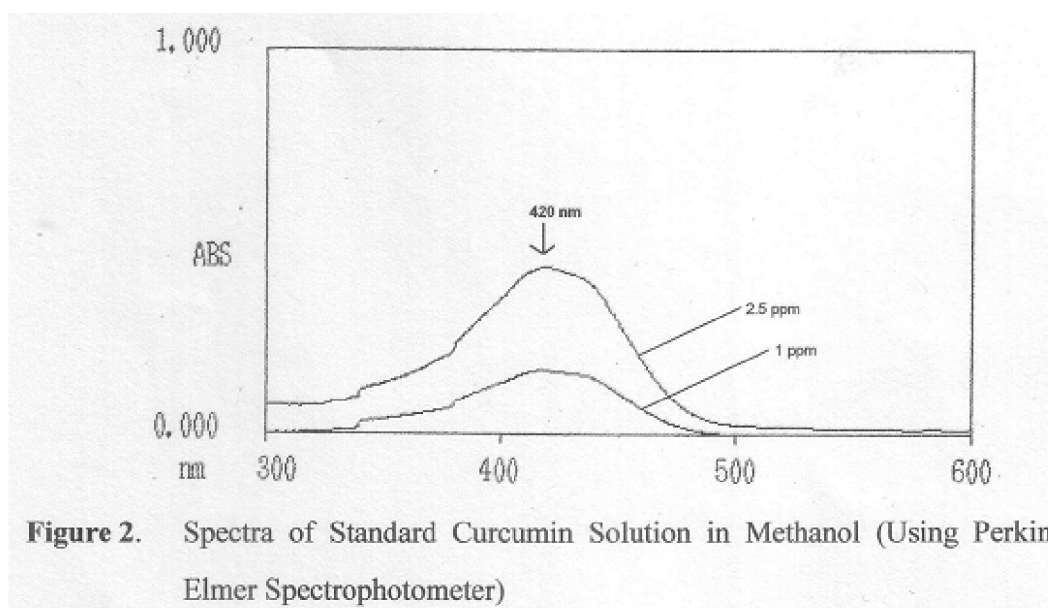


Figure 2. Spectra of Standard Curcumin Solution in Methanol (Using Perkin Elmer Spectrophotometer)

Acid glacial 1% (ratio: 45:55 v/v). The optimum eluent flow rate was 1.0 ml/minute.

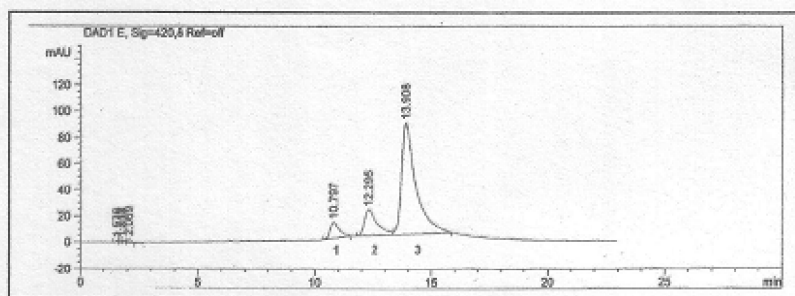
Figure 3 shows three peaks obtained from chromatogram of standard Curcumin with retention time (t_R) of 10.7, 12.2, and 13.9 minutes, respectively. According to Naoki et al (2003), standard Curcumin which is obtained by extraction from *Curcuma longa* generally contains BDC and DC. Percentage area of the third peak was 77.01%. It was in conformity with Curcumin's Certificate of Analysis that the third peak was Curcumin. The first and second peak were supposed to be BDC and

DC, respectively. Curcumin can be resolved from DC with R_s value of 1.73.

Figure 4 shows HPLC chromatogram of *Temulawak* capsule sample. That chromatogram shows two significant peaks, which have t_R of 12.3 minutes and 13.9 minutes respectively. The second peak was supposed to be curcumin based on its similarity of retention time (t_R), peak's profile, and peak's spectra profile (has matching factor value of 999.93) compared to standard Curcumin.

Chromatogram of sample extract that was spiked with standard Curcumin can be seen on Fig. 5. The supposed Curcumin's

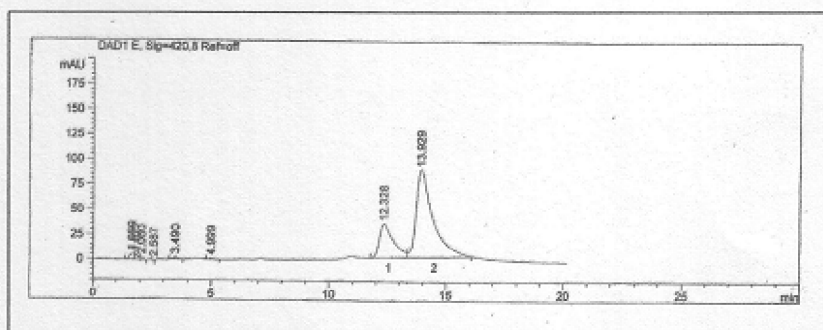
Signal
(mAU)



Retention Time (min)

Figure 3. Chromatogram of Standard Curcumin
1 = BDC, 2 = DC, 3 = Curcumin

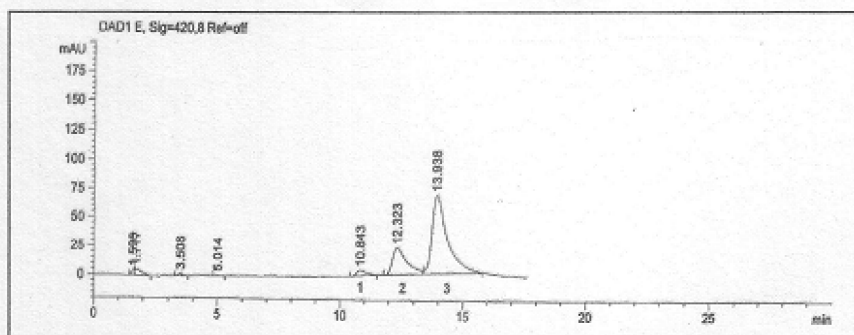
Signal
(mAU)



Retention Time (min)

Figure 4. Chromatogram of *Temulawak* Capsule Sample
1 = DC, 2 = Curcumin

Signal
(mAU)



Retention Time (min)

Figure 5. Chromatogram of Sample's Extract Spiked with Standard Curcumin
1 = BDC, 2 = DC, 3 = Curcumin

peak consistently showed its similarity of retention time (t_R), peak's profile, and peak's spectra profile compared to standard Curcumin.

Linearity

According to linearity test that had been established, Curcumin concentrations in the range of (23.56-62.83) $\mu\text{g/mL}$, showed a linearity correlation with its relative response of detector (peak area). Coefficient correlation ($r_{\text{calculated}}$) was 0.9997, more than r_{table} of 0.9000 (for $p = 0.95$ and $n = 5$). Linear regression equation was $y = 126.4 X - 1287.8$ with relative standard deviation of function (V_{x_0}) 0.99%.

Precision and Accuracy

Accuracy of the method was validated by spiking sample's extract with standard Curcumin at three concentration level 23.56, 39.27, and 47.12 $\mu\text{g/mL}$. Each concentration was analyzed triplicate. Recoveries of standard Curcumin concentration added were at the range of (93.60-118.17)% with CV 6.99%. The recoveries of standard Curcumin added were shown on table 1.

Instrument precision was determined by injecting 10 times of each 20,0 μL 50 $\mu\text{g/mL}$ standard Curcumin solution into HPLC system. The coefficient of variation (CV) of peak area observed (1.19%) showed the reproducibility of instrument in determining peak area of Curcumin was good. Precision of the method was studied by assaying nine samples spiked with standard Curcumin of 23.56, 39.27, and 47.12 $\mu\text{g/mL}$. The coefficient of variation (CV) of percentage of standard Curcumin recoveries 6.99% was good.

Analysis of the Sample

The concentrations of Curcumin in Temulawak capsule sample obtained were in the range of (8.836-8.995) mg as reported in table 2. The Curcumin concentration in sample was higher (177%) than that mentioned in its label (5 mg per capsule).

CONCLUSIONS

The method that was used for determining Curcumin content in pharmaceutical product (capsule formed) fulfilled with the standard validation criteria. The Curcumin content in each capsule sample was found to be (8.89 \pm 0.06) mg. It showed that samples contained higher amount of Curcumin than that claimed on its label, 5 mg.

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