CURCUMIN QUANTIFICATION IN DOSAGE FORMS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY*

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ABSTRACT

Curcumin quantification in dosage forms using high performance liquid chromatography (HPLC) has been carried out. This experiment was aimed to determine analysis conditions to be applied in curcumin dosage form analysis using HPLC. The experiment has been done with the following steps: mobile phase preparation, instrument preparation, working solution preparation followed by precision assay, accuracy assay, standard curve estimation, and sample measurement. The chosen condition was HPLC, LC-10AT, Shimadzu; column Shimadzu C_{18}, VP-ODS, 150L × 4.6; acetonitrile-2% acetic acid (45:55) as mobile phase; acetonitrile as solvent; detection at 420 nm; flow rate 1.2 mL/min. The result showed a correlation coefficient value was 0.9999, recovery value for accuracy assessment was 99.50%, variation coefficient for precision was 1.33%, limit of detection was 0.7816 ppm, and limit of quantification was 2.6053 ppm. Thus, the analytical method using HPLC for curcumin were feasible for curcumin quantification in dosage forms. This method has been applied for measuring three curcumin dosage forms A, B, C, and one curcumin raw material D. Samples A, B, C, and D measurement gave 105.86, 87.12, 10.71, and 130.35%, respectively.

INTRODUCTION

Curcumin is a principal curcuminoid of Indonesian spice turmeric. Curcumin is an orange-yellow coloring principle obtained by solvent extraction of turmeric. Pharmacology activities for curcumin are antibacterial, antifungi, antihepatotoxic, analgesic, antiinflammation, anticholesterol, choleretic, antioxidand, and so on. Cholagogum activity is marked by increase of bile production and secretion that worked choleretic and cholekinetic (Sidik 1992). By increasing bile secretion, it will decrease solid particles in gallbladder. This condition reduces bile cholic, stomach puffing caused by fat metabolism disorder, and lower blood cholesterol level (Dalimartha 2001). Curcumin and xanthorrizol are marker compounds in curcuma rhizomes (Badan POM 2004). Marker is a biological active substance marking or the major compound in a simplisia.

Research of dosage form with herbal drugs as raw material nowadays focused on isolation, identification, and pharmacological study of active substance, while quantitative analysis of active substance in herbal drug that might be unstable after distribution is rarely found. Curcumin quantitative analysis in 1983 were carried by high performance liquid chromatography (HPLC) method using Nucleosil-NH_2 column and reversed phase with ethanol as mobile phase, and fluorometer detector. Some curcuminoid analytical methods using HPLC such as mix of acetonitrile:acetic acid 5% (51:49) as mobile phase and ultraviolet detector, trifluoroasetic acid:water (40:60) and visible detector, acetonitrile:acetic acid 0,25% and visible detector, and acetonitrile:acetic acid 7,6% (55:45) with visible detector. The relative polar property of curcuminoid and consisting hydroxyl chromophore group makes its quantitative analysis able to be carried out by using reversed phase system with octadesilsilane column and ultraviolet-visible detector. Then this research carried out curcumin quantification in dosage forms using HPLC reversed phase system equipped with visible detector.

MATERIAL AND METHOD

Material

The materials used were curcuminoid (Sigma) and acetonitrile (JT Baker).

Equipment

HPLC, LC-10AT, Shimadzu; Column: Shimadzu C_{18}, VP-ODS 150L × 4.6; Membrane filter 0.45 μm; Syringe filter 0.45 μm.

Determination of Analysis Condition

a. Mobile phase preparation

1. An amount of 20 mL glacial acetic acid measured then put into 1 L beaker glass; 980 mL aqua bidestilata was the added. The mixture was stirred using magnetic stirrer for 10 minutes to obtain homogeneous solution. The solution was then filtered by pump filter; the filtrate was placed in Erlenmeyer flask and degassed. The solution was then placed into mobile phase bottle and labeled FG-Ac 2%.
2. An amount of 1 L acetonitrile was placed in beaker glass. The solution was filtered by pump filter then placed in mobile phase bottle and labeled FG-ACN.

b. Instrument Optimation

HPLC column was cleaned up by elution, filtration, and degasion. Elution runs for 1 hour, then the column was washed using acetonitrile for 1 hour. After the washing step, the column was

conditioned by eluting mobile phase 2% acetic acid and acetonitrile (45:55) for 30 minutes and at the same time it was run for baseline.

c. Working solution preparation

For standard solution 100 ppm, 50 mg curcuminoid standard was weighed accurately, diluted in 50 mL volumetric flask until the limit mark with acetonitrile, then 2.5 mL was taken and diluted into 25 mL volumetric flask with the same solvent. Procedures for other standard concentrations were done similarly.

d. Linearity and Range Study

Six concentrations of curcuminoid standard solution were made: 5, 10, 20, 40, 80, and 100 ppm. Each solution was filtered by syringe filter 0.45 µm. The solutions were injected into injector once for each time and the area under curve was recorded and measured for the r^2 and r values.

e. Presicion Study

Ten solutions of 20 ppm curcuminoid were made for standard solutions. Each solution was filtered by syringe filter 0.45 µm. The solutions were injected into injector once for each, and then the area under curve was recorded and measured for the coefficient variation values (CV≈ 1).

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f. Accuracy Study

Three concentrations of curcuminoid standard solution were prepared for 20, 40, and 80 ppm. Each solution was filtered by syringe filter 0.45 µm. The solutions were injected into injector three times for each silution and then area under curve was recorded and measured for the average recovery values.

g. Limit of Detection and Limit of Quantification Study

Six concentrations of curcuminoid standard solution were prepared: 5, 10, 20, 40, 80, and 100 ppm. Each solution was filtered by syringe filter 0.45 µm. The solutions were injected once for each time and then the area under curve was recorded and measured for the standard deviations, limit of deviation (LOD), and limit of quantitation (LOQ) values.

h. Standard curve estimation

Six concentrations of curcuminoid sandard solution were prepared: 5, 10, 20, 40, 80, and 100 ppm. The solutions were injected into injector once for each and the area under curve was recorded and measured for the correlation coefficient in linear regression equation ($Y = ax + b$).

Quantitative Analysis

a. Sample solution preparation

1. Dosage form sample. Two samples of tablet were weighed and powdered to homogeneous. The powder sample was weighed for 1 dosage and diluted into 25 mL volumetric flask; 5 mL

solution was taken and diluted with the same solvent into 10 mL in a volumetric flask. The solution was filtered with 0.45 µm Whatman paper, and placed into HPLC vials. For capsule dosage form, similar steps were carried out as for tablet, with adjusted dilution for curcuminoid concentration in sample.

2. Curcuminoid raw material sample. Fifty mg curcuminoid sample was weighed accurately, diluted in 50 mL volumetric flask with acetonitrile, and then 2.5 mL of the solution was taken and diluted into 25 mL volumetric measure flask with the same solvent. The solution was filtered with 0.45 µm Whatman paper, and placed into HPLC vials.

b. Concentration measurement

Eighty ppm solution from the stock solution of four samples was injected into injector twice for each sample. The area under curves were recorded and plotted into standard curve linear regression equation.

RESULTS AND DISCUSSION

The chosen experiment condition obtained for HPLC (LC-10AT, Shimadzu. Column: Shimadzu C_{18}, VP-ODS 150L × 4.6) was using mobile phase consisted of acetic acid 2% and acetonitrile 55:45. The chosen solvent was acetonitrile. The curcumin linear regression curve was $y = 36300x - 2771.6$, with correlation coefficient $r = 0.9999$. Recovery results at nine concentrations measured in this experiment gave 99.50%. The presicion study resulted from ten concentrations measured in this experiment gave variation coefficient of 1.33%. LOD and LOQ study gave 0.7816 ppm and 2.6053 ppm, respectively.

Sample Quantitative Analysis

From three dosages form samples and one curcuminoid raw material samples were as follows: average concentration of A: 105.86%, B: 87.12%, C: 10.71%, and D: 130.35%.

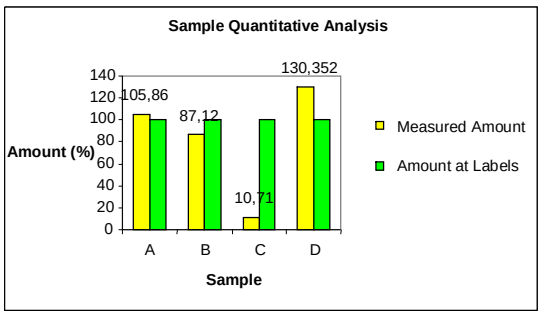

Figure 1 Results of Sample Quantitative Analysis

Comment [F3]: Hapuskan garus dalam keterangan legenda.

The method used in this experiment refers to Yang *et al.* (2006) experiment, however some conditions such as mobile phase, flow rate, and injection volume were not applied to this experiment. They carried out curcumin analysis in Gan Zhi Ping capsules using Zorbax C_{18} column, mobile phase acetonitrile-7,6% acetic acid (55:45), flow rate 1mL/minute and injection volume 10μL, while the detection run at 420 nm. We conclude that the use of this method does not guarantee a good accuracy and precision when applied at different place and condition. Therefore, it is necessary to run an analytical validation method. Analytical validation method is an observation on certain parameters, based on laboratory experiment to prove that those parameters meet their requirements. However, a valid method in a certain condition is not a guarantee to be valid in other conditions.

The mobile phase used in this experiment was mixture of acetonitrile and 2% acetic acid. This is results of several trials until we got the best condition at 2% acetic acid for composition (45:55) with acetonitrile. Curcumin was observed as the last peak eluted. Tonnesen and Karlsen (1983) reported that curcuminoid from turmeric eluted at the first peak was bidesmethoxycurcumin, followed by desmethoxycurcumin and curcumin. The method validation from the chosen conditions was started with standard curve estimation. The standard curve made from six concentrations of standard solutions at the range of 5 to 100 ppm gave a correlation coefficient (*r*) 0.9999. This value is taken from the third peak of chromatogram which was assumed as curcumin, as observed in the previous experiment and based on the largest area under curve considering curcumin as major compound of curcuminoid.

Accuracy was estimated by recovery study using direct comparation with the standard. The study used three different concentrations of standard solutions, which is 20, 40, and 80 ppm, with three replications for each measurement. The recovery value was 99.50%. This value meets the requirement for accuracy assay, which must at the range of 98-102%. Presicion was estimated by measuring ten standard solutions with the same concentration (20 ppm). From ten solutions measured, the coefficient value was 1.33%. This value also meets acceptance criteria for precision assay, which must below 2%.

Curcumin Quantitative Analysis in Samples

Samples in this experiment were three curcuminoid dosage form samples and one curcuminoid raw material sample. Sample A, B, and C are the dosage form samples, while sample D is raw material sample. Selection of these samples was run randomly to dosage forms with curcuminoid as active ingredient, whether as single ingredient or in a mixture with any ingredients, with variation of dose and indications. Raw material sample selection is based on consideration of

method application on curcuminoid isolation from nature material. This raw material estimation may be useful for small industry that does not own analytical method for curcumin quantitative analysis.

Each samples then measured twice. Sample A measurement gave curcumin concentration 105.86% from its real value on the label (100%). Higher concentration may caused by inaccurate production or tolerancy from analytical method. Curcumin concentration in sample B was 87.12%. It might be caused by inaccurate production or curcmin degradation during distribution and storage. However, the incorret value still meets the requirement range. For sample C, the result was only 10.71% curcumin. This value is far away from its real value on the label and did not meet the requirements. The deviation that very far from the true value may cause a different indication. Sample D showed curcumin concentration of 130.35% from the label. This value is far above its true concentration on the label, which was 80%. It might caused by improper isolation procedure and quantitative analysis on label, and also tolerancy from the method.

CONCLUSIONS

From the results above we can conclude that curcumin can be measured by HPLC reversed phase system, using C_{18} column; acetonitrile-2% acetic acid as mobile phase in a ratio of 45:55 and acetonitrile as solvent. Accuracy and precision is fulfilled by 99.50% for recovery, and variation coefficient for presicion is 1.33%. Whereas the limit of detection was 0.7816 ppm, and limit of quantification was 2.6053 ppm. With this analytical method, curcumin concentration in samples can be determined for samples A, B, C, and D (from the concentrations written in the label) as 105.86, 87.12, 10.71, and 130.35%, respectively.

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Comment [F4]: perlu dicek antara acuan dalam teks dan yang didaftarkan berikut ini

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