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Original Article

VALIDATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR DETERMINING THE LEVELS OF CURCUMIN IN TABLET PREPARATIONS

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ABSTRACT

Objective: The purpose of this research was to optimize and validate HPLC method for determining the curcumin content of tablet preparations in less than ten minutes.

Methods: Curcumin can be analyzed qualitatively or quantitatively using reverse phase HPLC equipped with an isocratic elution system. Furthermore, this research used a visible detector, which contained octadecyl (C18) in its stationary phase at a wavelength of 428 nm and a mobile phase of acetonitrile-acetic acid-aquabides (60:1:39) mixture with a flow rate of 1.0 ml/minute. The chromatographic setup can separate curcuminoids, such as bisdemethoxycurcumin, demethoxycurcumin, and curcumin in less than 10 min. The method is valid on the basis of its selectivity, linearity, accuracy, and precision.

Results: The results showed that there was a high degree of selectivity with a resolution value (Rs) of 1.670 and a high degree of linearity with a correlation coefficient (r) of 0.9915 over a range of 1.38-2.04 µg/ml. Curcumin had a recovery test value of 100.73±1.04%. The curcumin content of the tablet preparations by producing A and B were 3.99±0.02% and 3.97±0.01%, respectively.

Conclusion: According to the results, HPLC is a valid method for determining curcumin levels in tablet preparations in accordance with ICH specifications (International Conference on Harmonization).

Keywords: Validation, Curcumin, Tablets, HPLC

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INTRODUCTION

Hepatitis B virus (HBV) infection International endemic in Indonesia at a moderate to a high level [1] due to the lack of an optimal therapy up to this point, which has caused experts to exploit alternative therapies such as herbal medicines. Curcumin is one of the compounds frequently used to treat hepatitis disorders due to its hepatoprotective properties [2]. Furthermore, it is available in capsules, tablets, syrups, and suspensions. Regular quality control tests are necessary to ensure the marketability of drugs and the reproducibility of preparation production [3], such as quantitative determination or assay using UV spectrophotometry [4]. The curcumin concentration in temulawak or Curcuma tablets and standardized herbal medicinal liquid preparations were determined using TLC densitometry and high-performance reverse-phase liquid chromatography, respectively. The high performance reverse-phase liquid chromatography employed a gradient system with a mobile phase, which composed of 2% (90:10) glacial methanol-acetic acid [5, 6]. Additionally, curcuma extract was analyzed qualitatively and quantitatively using a mobile phase of acetonitrile, acetic acid, and aquabidest (50:1:49) [7]. Curcumin levels in tablets can be determined using HPLC method, which has been extensively used to discover and develop new analytical methods. HPLC is a common method of drug analysis due to its selectivity, simplicity, and high sensitivity, which are ideal for analyzing a wide variety of drugs in biological preparations and fluids [8]. Furthermore, this method was chosen over other analytical techniques due to its higher degree of sensitivity, selectivity, separability and ability to detect trace amounts of analyte [6].

Previously, HPLC method for analysing the concentration of curcumin in tablets was validated using gradient elution and a mobile phase mixture, which consists of sodium acetate and acetonitrile buffer [9]. Curcumin was extracted from plasma in another research using an isocratic technique on column C18 Kromasil® 100-5 (250 x 4.6 mm, 5m) and a mobile phase consisting of acetonitrile-methanol-aquabidestilata-acetic acid (33:20:46:1) [8]. Additionally, other research employed a mobile phase, which consists of 2% acetonitrile-acetic acid (40:60) solution at a flow rate and retention time of 1.3 ml/minute and 16.72 min, respectively [10]. Another research aimed to validate HPLC method in determining the curcumin concentration in liquid preparations using a mobile phase, which consisted of 2% (90:10) methanol-glacial acetic acid at a flow rate and retention time of 0.5 ml/minute and 6.00 min, respectively [6]. Although this research was efficient, it was ineffective due to the inability of the model to separate curcuminoids. Subsequently, the retention time of curcuminoids was determined in another research using HPLC and a mobile phase containing acetonitrile-acetic acid-aquabides (50:1:49) [7]. The aforementioned research was effective but still inefficient since it consumed a significant amount of mobile phase with a relatively long retention time at this flow rate. Therefore, this research aimed to optimize and validate HPLC method for determining the curcumin content of tablets in less than 10 min of analysis. The mobile phase composition was modified to acetonitrile-acetic acid-aquabides (60:1:39), while the flow rate was varied between 0.8, 1.0, or 1.2 ml/minute. Separation of curcuminoid compounds was performed to determine the quantity and quality of curcumin in the preparations.

MATERIALS AND METHODS

Chemicals and reagents

The materials used in this research included curcumin comparison standards (obtained from Merck in Darmstadt, Germany), 20 mg curcumin tablets (manufactured by Factory A), 13.3 mg curcumin tablets (manufactured by Factory B), and a placebo mixture of curcumin tablets, as well as HPLC grade methanol, acetonitrile, and glacial acetic acid (obtained from Mallinckrodt in Phillipsburg, USA). The equipment included a high-performance liquid chromatography system (Shimadzu Nexera LC-20 AD, Richmond, USA) equipped with a visible detector (Shimadzu SPD-20A, Richmond, USA), a 4.6 x 250 mm x 5 µm column (Reliant® RP-18e from Dublin in Ireland), an ultrasonic sonicator (Branson 8510 from Darmstadt in Germany), a mobile phase filter equipped with a vacuum (Welch, Sk from Pyrex in Alexandria, Australia).

Preparation of curcumin standard stock solution

About 10 mg of standard curcumin was carefully weighed, incorporated into a 10 ml volumetric flask and then diluted with methanol pH 4 to obtain a concentration of 1000 g/ml. A total of 1.0 ml of this solution was transferred to a 10 ml volumetric flask and diluted with methanol at a pH 4 to obtain a concentration of 100 g/ml. Subsequently, 100 µl of the solution was transferred to a 10 ml volumetric flask and diluted with methanol at a pH 4 to a concentration of 1.0 µg/ml.

Preparation of simulated tablet powder

A mixture of simulated tablet powder equivalent to 20 curcumin tablets (placebo) was prepared using various analytes at 80% (16 mg), 100% (20 mg), and 120% (24 mg). Composition of placebo (tablet matrix):

Aerosil 0,5%

Magnesium stearate1%

Avicel pH 102 to 400 mg

Optimization of high-performance liquid chromatography method

Determination of the maximum wavelength of curcumin

Standard stock solutions of 100 µg/ml curcumin in total volumes of 40, 100, and 160 ml were diluted with methanol at a pH of 4 in a 10 ml volumetric flask to obtain concentrations of 0.4, 1.0, and 1.6 µg/ml. The maximum wavelength value obtained is then used to calibrate the detection wavelength of HPLC system.

Selection of the mobile phase composition

The optimal mobile phase composition in previous research [7] was used as the starting point for selecting the composition of the mobile phase, with initial conditions consisting of a mixture of acetonitrile-acetic acid-aquabidest (50:1:49). Subsequently, the composition of the mobile phase was changed to acetonitrile-acetic acid-aquabidest (60:1:39).

Flow rate selection

About 20 µl of a standard stock solution consisting of 1.0 µg/ml curcumin, which had been filtered using porous filter paper and sonicated, was injected into HPLC using the selected mobile phase. The flow rate used was 0.8 ml/minute, which was then varied to 1.0 ml/minute and 1.2 ml/minute. Furthermore, the retention time (tR), tailing factor (Tf), several theoretical plates (N), and HETP were used to determine the flow rate that produced the most effective and efficient separation.

System conformity test

Determination method

About 20 µl of a standard stock solution containing 1.0 µg/ml curcumin, which had been filtered with porous filter paper and sonicated, was injected five times into the chromatograph. Each standard solution produced a chromatogram with a relative standard deviation of the retention time and a peak area of ≤ 2.0% [10].

Determination of the calibration curve

Several standard solutions of curcumin were prepared at eight different concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 µg/ml from the standard stock solution at 100 µg/ml in a 10 ml volumetric flask. Methanol at a pH of 4 was added up to volume before the solution was filtered with a porous filter paper and sonicated.

Curcumin analysis method validation

Selectivity test (specificity)

Determination method: A 1:1 mixture of the sample solution and 20 µl standard stock solution containing 1.0 g/ml curcumin was filtered through a porous filter paper, sonicated, and injected into the chromatogram to determine the peak area. The chromatogram was used to determine the selectivity value of each analyte peak.

Linearity test, detection limit, and quantitation limit

Various commercial tablet samples were used to conduct the Linearity test, which determined the relationship between the concentration and peak area using six solutions at different concentrations.

Precision and accuracy test

The recovery test was conducted by adding standard curcumin to the placebo (tablet matrix) to obtain an analyte content of 80, 100, and 120 %, respectively, based on the Spiked Placebo Method.

Determination of precision and accuracy

About 20 µl of curcumin sample solution was filtered through a porous filter paper, sonicated, and injected into the chromatograph. Furthermore, this was conducted three times for each concentration to produce a chromatogram. The analyte content, CV value and % recovery were determined from the peak area.

Determination of the curcumin content in tablets from producers A and B

Preparation of the solution

About 20 curcumin tablets were weighed carefully to determine the average weight before they were powdered. The curcumin tablet powder was carefully weighed equivalent to a concentration of 1 mg and placed in a 10 ml volumetric flask. Subsequently, it was dissolved in methanol at a pH of 4 up to volume and shaken homogeneously. 100 µl of the solution was then placed in a 10 ml volumetric flask and diluted with methanol at a pH of 4 up to volume to obtain a concentration of 1.0 µg/ml.

Determination method

About 20 µl of the sample solution was filtered through a porous filter paper, sonicated, and injected into the chromatograph. Subsequently, the peak area of the chromatogram and the concentration of the sample solution were determined.

RESULTS AND DISCUSSION

Determination of wavelength

The wavelength was determined in order to detect curcumin in an HPLC assay using a UV detector. The result showed that the highest absorption occurred at a wavelength of 428 nm (fig. 1) by creating an absorption profile at three different concentrations using a spectrophotometer. The wavelength was determined by comparing the three concentrations to the theoretical wavelength of 430 nm [6]. The wavelength of the measured results should not be more than±2 nm to the theoretical wavelength. Additionally, it is used as a detector for HPLC.

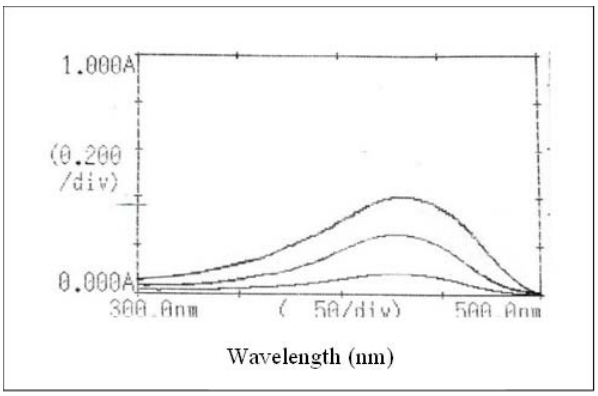

Fig. 1: The maximum absorption wavelength of curcumin solution of 0.4; 1.0; 1.6 µg/ml

Determination of the optimum HPLC conditions

The mobile phase was optimized because its composition has an effect on the effectiveness and efficiency of chromatography. Curcuminoids containing bisdemethoxycurcumin, demethoxycurcumin, and curcumin were used as the reference standard.

The mobile phase of 2% methanol-glacial acetic acid

The mobile phase was prepared at a concentration of 90:10, and a retention time of approximately±6 min was obtained. The mobile phase was tested at three different flow rates, including 0.8, 1.0, and 1.2 ml/minute. However, this mobile phase could not separate curcuminoid compounds, specifically bisdemethoxycurcumin, demethoxycurcumin, and curcumin, since the polarity was insufficient, as indicated by the chromatograph reading only one peak in fig. 2.

The mobile phase of acetonitrile-acetic acid-aquabides

The mobile phase was acetonitrile-acetic acid-aquabides (50:1:49) with a flow rate of 1.0 ml/minute. Three peaks corresponding to curcuminoid compounds were obtained, which identified the presence of bisdemethoxycurcumin, demethoxycurcumin, and curcumin. However, the retention time was±16 min, which exceeded the target retention time of less than 10 min (fig. 3).

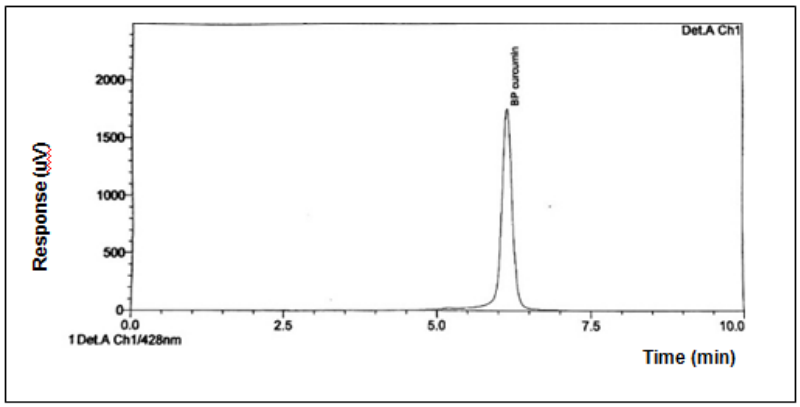

Fig. 2: Chromatogram of standard of curcumin with 2% methanol-glacial acetic acid as the mobile phase (90:10)

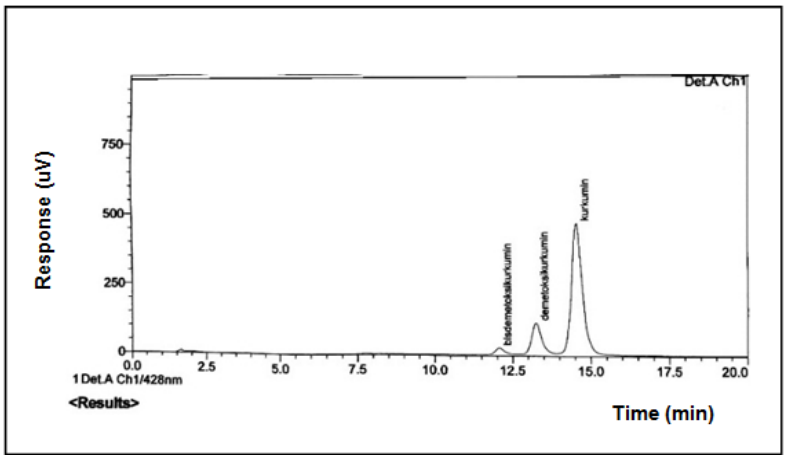

Fig. 3: Chromatogram of standard of curcumin with acetonitrile-acetic acid-aquabides (50:1:49) mobile phase with a flow rate of 1.0 ml/min

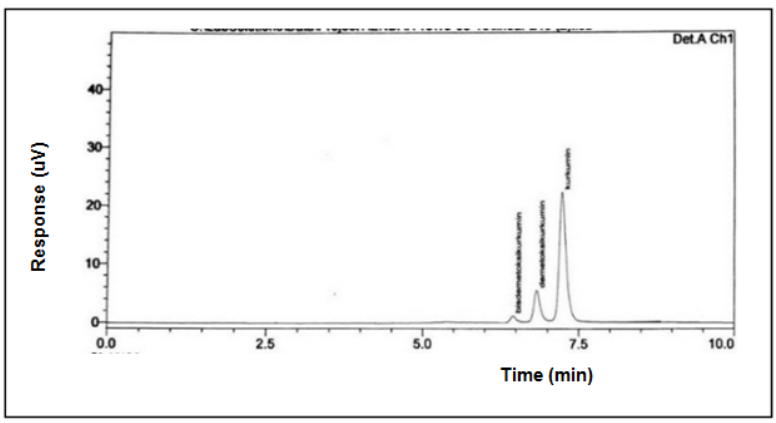

Fig. 4: Chromatogram of standard of curcumin with acetonitrile-acetic acid-aquabides (60:1:39) mobile phase and a flow rate of 1.0 ml/min

The concentration of the mobile phase was varied using the same mobile phase, acetonitrile-acetic acid-aquabides (60:1:39). However, there was a higher distribution of curcumin in the mobile phase when the polarity was reduced. Therefore, the curcumin will be released more rapidly and the separation will be improved (fig. 4).

A flow rate of 0.8 and 1.2 contributed to the retention time of±7.5 and±7 min, respectively, which were comparable to a flow rate of 1.0. Therefore, the flow rate was set to 1.0 since a lot of mobile phases were required while using a higher flow rate, which reduces the efficiency.

The wavelength, mobile phase, and flow rate were all selected carefully to produce a good chromatogram that could be used to ensure that separation could be improved and fulfills pharmacopeial standards [10], while the resolution is > 1.5. Also, the tailing factor of<1.2 indicates that the peak is more symmetrical. However, the column is more efficient if N<1500 at a shorter retention time. According to these results, HPLC conditions similar to those shown in table 1 were used for further experiments.

Table 1: Selected HPLC conditions

HPLCparameters	**Selected HPLC conditions**
Injection volume	20 µl
Silent phase	Column Reliant® RP-18e (4.6x250 mm; 5 µm)
Mobile phase	acetonitrile-acetic acid-aquabides (60:1:39)
Flow rate	1.0 ml/minute
Detector	UV 428 nm

Results of system conformity test

The results of the system conformity test are shown in table 2, along with the peak area obtained after five injections. The system conformity of SBR for the retention time, peak area, resolution, and column efficiency were 0.26%, 1.04%, 1.6%, and 0.51%, respectively. Additionally, this value complies with the 2.0% SBR requirements of the fifth edition of the Indonesian Pharmacopoeia [10]. Therefore, the conditions for employing HPLC in the quantitative analysis of curcumin in tablets have led to the development of a unified system that can provide high-quality results.

Table 2: Results of the system conformity test

No	Parameters	N	Mean	CV	CV (%)
1	Resolution	5	1.666	0.024	1.456
2	Column efficiency	5	2296.85	11.81	0.514
3	*Tailing factor*	5	1.143	0.001	0.114
4	Retention time(min)	5	7.189	0.018	0.256
5	Peak area	5	280379	2914	1.039

Calibration curve

The standard calibration curve for comparing the various curcumin concentrations was developed by weighing a single concentration of the curcumin stock solution. Subsequently, the curcumin concentration (µg/ml) was constructed as the x-axis, while the peak area as the y-axis in order to derive the calibration curve, which was used to calculate the assay.

According to the standard calibration curve for comparing the levels of curcumin in fig. 5, the regression line equation y = 56517.4972+230912.2x was obtained with a correlation coefficient (r) of 0.9904, which indicate that the concentration of curcumin is directly proportional to the peak area between a concentration of 0.4-3.22 g/ml.

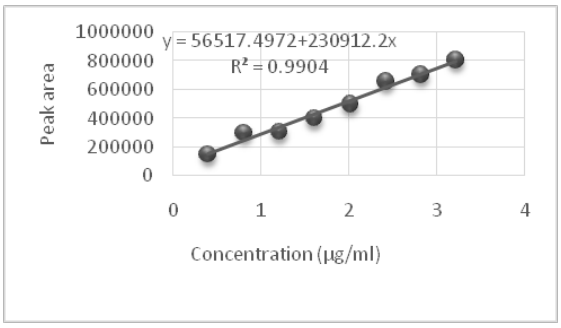

Fig. 5: Standard calibration curve for curcumin comparison

Linearity test results, range, detection limit, and quantity limit

Linearity test and range

The Linearity test was conducted by determining the effect of the curcumin sample matrix on linearity. Fig. 6 showed the result using the regression line equation y = 27584.5680+260110.08x, with a correlation coefficient (r) of 0.9915, which is linear within the range of 1.38-2.04 g/ml. There is a linear relationship between the concentration and peak area of curcumin when the correlation coefficient is greater than 0.999. The range indicates that the lower and upper limits of the analyte can be determined with sufficient accuracy, precision, and linearity.

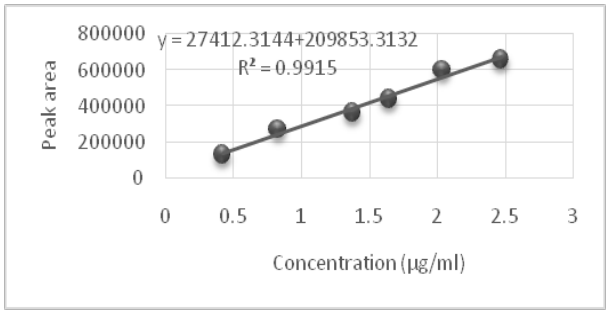

Fig. 6: Curcuminoid linearity test curve

Determination of the detection and quantitation limit

The detection limit test determines the lowest concentration of curcumin that can still be detected using a sample of 0.4136 µg/ml. While the quantitation limit test provides information on the lowest concentration of curcumin in the sample that can be determined with an acceptable degree of precision and accuracy, using a sample of 1.2532 µg/ml. The quantitation limit can be used to determine the lowest concentration of curcumin that should be prepared for injection into the chromatograph. Therefore, the concentration of curcumin used in linearity, precision, and accuracy tests should be greater than the quantitation limit values obtained.

Test results of the accuracy and precision

The accuracy and precision tests were conducted using the placebo spike method, which incorporated several analytes into the carrier mixture (placebo). Subsequently, the mixture was analyzed and the results were compared. This method was chosen since the matrix of the placebo sample was known. Accuracy shows the degree of

closeness between the analysis results and the actual analyte concentration. Accuracy is expressed as a percentage of the added analyte recovered. Curcumin recovered at a rate of 100.73±1.04% (98.0-102.0%), which fulfilled the requirements (11). Therefore, accurate analysis (accuracy) can obtained regardless of the amount of analyte added, specifically when HPLC method is used under the optimized conditions. Precision is a measure of the repeated similarity between analyte sizes, which is expressed as CV. The precision test consisted of three repetitions at various concentrations, which yielded CV of 1.04%.

Comparison with other research

Table 3 compares the results of this research to those of other studies. The sample tested was a curcuma extract, which was consistent with the literature [7]. Meanwhile, literature [6, 12, 13] was a mixture of curcuma and other compounds, whereas literature [6] was specifically Silymarin. The advantage of this method is that the mobile phase is more efficient than a buffer solution with a low pH [12, 13], which can damage the column over time and lead to a decreased column durability due to the nature of the phosphate buffer. Also, the use of buffer during column washing is time consuming, which makes it less efficient. HPLC system can be used in any type of analysis since a gradient system and a simpler elution system were used, such as the isocratic elution system [6, 12]. This method was more selective than those described in the literature [6, 13], since it can separate curcuminoid compounds into bisdemethoxycurcumin, demethoxycurcumin, and curcumin depending on the polarity of the mobile phase used. The retention time is significantly shorter than what has been reported in the literature [7]. Therefore, the time required to read the analyte is reduced and the system requires a small amount of mobile phase, which saves both time and resources.

Table 3: Comparison of method performance with other research

Parameter	Results	Literature			
		(12)	(13)	(6)	(7)
Sample matrix	Tablets	Tablets and capsules	Polymeric microparticles	Syrup	Extract
Mobile phase	Acetonitrile-acetic acid-aquabides pH 4.01 (60:1:39)	KH_2PO_4 solution 0.05 M pH 2.3-methanol-acetonitrile	Acetonitrile-phosphoric acid 0,1%-methanol (50:40:10)	Methanol-glacial acetic acid 2%(90:10)	Acetonitrile-acetic acid-aquabides (50:1:49)
Elution system	Isocratic	Gradient	Isocratic	Gradient	Isocratic
The peak of curcuminoids	Separated	Separated	Inseparable	Inseparable	Separated
Retention time (min)	±7	±36	±11	±6	±15
LOD (µg/ml)	0.41	10.4	0.084	-	-
LOQ (µg/ml)	1.25	11.8	0.129		
Range (µg/ml)	1.38-2.04	75-175	0.5-20	-	-

Determination of the curcumin levels in tablet preparations

The levels of curcumin in the tablet preparations were determined using HPLC, which has been validated as a quality control method for analyzing curcumin tablet preparations [14, 15]. Furthermore, this procedure was repeated six times using the same concentration. The assay results from two different manufacturers obtained average curcumin levels of 3.99±0.02 and 3.97±0.01% in tablet preparations of 20 mg from factory A and 13.3 mg from factory B, respectively, after six repetitions at the same concentration.

CONCLUSION

The concentration of curcumin in tablet preparations can be determined in less than 10 min using reverse phase HPLC with octadecyl (C18) as the stationary phase, acetonitrile-acetic acid-aquabides (60:1:39) as the mobile phase with a flow rate of 1.0 ml/minute and a 428 nm UV detector with a recovery of 100.73±1.04%, which fulfills the ICH requirements (International Conference on Harmonization).

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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