Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 44 / Number 1 / March 2024 / pp. 1-8

RP-HPLC Method Development for Determination of Curcumin in Commercial Turmeric Capsules

Duygu ENEŞ[1]*
ORCID: 0000-0002-8908-3177
Mustafa ÇELEBIER[2]
ORCID: 0000-0001-7712-5512
Derya DIKMEN[3]
ORCID: 0000-0003-2099-2863
Sacide ALTINÖZ[1]
ORCID: 0000-0003-2864-9218

[1]Başkent University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey

[2]Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey

[3]Hacettepe University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara, Turkey

Corresponding author:
Duygu ENEŞ
Baskent University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey
E-mail: duyguenes@baskent.edu.tr
Tel: +90 (312) 246 66 66

Received date : 20.02.2023
Accepted date : 05.12.2023

DOI: 10.52794/hujpharm.1253858

ABSTRACT

Curcumin is a natural polyphenol product isolated from the rhizome of the Curcuma longa plant and is the most active ingredient of this plant. Curcumin is hepatoprotective, nephroprotective, anti-thrombotic, cardioprotective, anti-diabetic and anti-rheumatic. The aim of this study was to develop an HPLC method for the analysis of curcumin in commercially available capsules. Supelco Discovery 5 µm C18 Column (4.6mm x 5.0 µm) was used for chromatographic separation. The mobile phase was acetonitrile (ACN), 20 mM phosphate buffer, pH 6 (40:60 v/v). The flow rate was 1.5 mL min-1. UV detection was at 262 nm. The retention time was about 6.13th minutes for curcumin. The developed method has been validated according to ICH guidelines. The method was found to be selective under the experimental conditionsIt was linear over the range 0.05 - 50.0 µg mL-1. The intraday and interday studies showed that the RSD % was less than 2.0 for intraday and 2.0 for interday, and the Bias% was less than 2.0 for intraday and 2.0 for interday. The method could be successfully used in curcumin-loaded commercial capsules' quality control.

Keywords: Curcumin, Turmeric, HPLC, Validation, Determination

1. Introduction

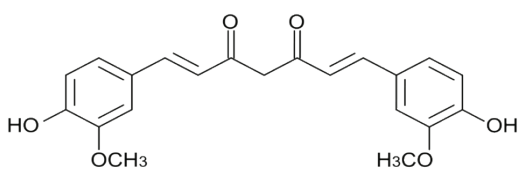
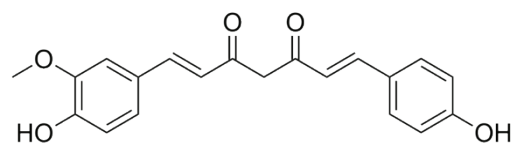
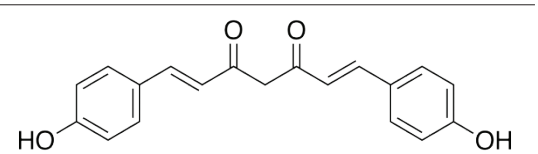
Curcumin (diferuloylmethane) is the orange-yellow component of turmeric or curry powder. It is a natural polyphenol product isolated from the rhizome of the plant *Curcuma longa*. [1]. Curcumin, which makes up 2-5% of the spice, is the most active component of turmeric [2]. Curcumin is a diferuloylmethane molecule containing two ferulic acid residues linked by a methylene bridge. Another synonym is 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione.) [3]. The molecular weight of curcumin, whose molecular formula is $C_{21}H_{20}O_6$, is 368.38 g/mol. Curcumin is a lipophilic orange/yellow crystalline powder, meaning it is insoluble in water but soluble in organic solvents such as ethanol, acetone and dimethylsulfoxide [4, 5]. Besides curcumin, *Curcuma longa* contains demethoxycurcumin and bis-demethoxycurcumin, which are called curcuminoids [6]. The main bioactive component of these curcuminoids is curcumin [7]. Table 1 shows the chemical structures of curcumin, demethoxycurcumin and bis-demethoxycurcumin.

Phytochemicals are found naturally in plants. Phytochemicals are used due to their diverse medicinal uses [8]. Medicines derived from plants are readily available, cheaper, safer and more effective, and rarely have side effects [9].

Curcumin has anti-inflammatory properties. It inhibits the NF-kappa B activation pathway [10]. In a study (2009), Liang et al found an anti-inflammatory effect of curcumin by inhibiting TNF-α, IL-1β and IL-6 [11]. It has also been shown to inhibit the growth of Moser cells in colon cancer by activating PPAR{gamma} [12]. Durgaprasad et al. were conducted to evaluate oral curcumin's effect on oxidative stress markers in patients with tropical pancreatitis. Lipid peroxidation has been shown to be reversed by curcumin [13]. Another study in 2006, evaluated the efficacy of curcumin as a maintenance therapy in patients with quiescent ulcerative colitis. Curcumin was shown to maintain remission [14]. Phan et al. were found that curcumin strongly inhibited hydrogen peroxide-induced damage in human keratinocytes and fibroblasts, and was therefore effective in wound healing [15]. Curcumin (found in turmeric) has been used to treat skin, lung and gastrointestinal problems, pain, wounds, sprains and liver problems since Ayurveda (1900 BC) [16]. Turmeric has anti-inflammatory, antioxidant, chemopreventive, chemotherapeutic, antimicrobial, antiviral, antifungal, anticarcinogenic, chemosensitive, radiosensitive and wound-healing activities. In addition, curcumin has hepatoprotective and nephroprotective, thrombosis-suppressing, anticardiac, antidiabetic and antirheumatic effects [17-19]. Moreover, individuals who regularly consume turmeric in their diets are significantly less affected by Alzheimer's disease [20].

The rhizomes of the *Curcuma longa* plant contain curcumin [20]. Curcumin supplements come from powder, liquid extract, or capsules containing tinc-

Table 1. Chemical structure of curcumin and its congeners.

Molecule name	Chemical structure
curcumin	
demethoxycurcumin	
bis-demethoxycurcumin	

tures [21]. There are also pills, lozenges, band-aids, and creams commonly available on the market [22]. The poor bioavailability of curcumin has limited its use to some extent. The route of administration, rapid metabolism, and elimination route, especially at low serum and tissue curcumin levels, reduce curcumin bioavailability [19]. New drug delivery systems are overcoming this obstacle. Curcumin is available in the form of supplements by combining it with substances that increase its bioavailability [21]. Bioavailability is increased with nanoparticles, liposomes and defined phospholipid complexes [19].

A dietary supplement is expected to contain the ingredients listed on the label in the specified amounts. This situation occurs only sometimes the case when there is poor quality control in the manufacture and storage of dietary supplements. There is also a lack of a comprehensive testing programme for dietary supplements [23]. Pesticides, heavy metals, mycotoxins and microbiological contaminants such as bacteria, mould or yeast are the most important contaminants of plant raw materials. Some regulations limit the content of contaminants to technically achievable and toxicologically acceptable levels. To measure such contaminants advanced analytical method is needed [24]. In the regular use of curcumin capsules, quality control of the relevant capsules is required, and as a purpose of this study, an analytical method was developed to analyze commercial curcumin capsules. Among these capsules, the capsule model, which is readily available in the market and obtained curcumin by extracting turmeric, was chosen.

In the literature research, there were validated methods for the determination of curcumin in potential extracts [25-27]. However, the aim of this study is to develop an analytical method to analyse curcumin in curcumin extract capsules. Thus, we developed an analytical method to determine a model for commercial curcumin capsules, and the method was proposed for other turmeric extract-containing capsules. This method could be used by supplement quality control laboratories, private laboratories and university researchers and has been validated and contributed to the literature.

2. Material and Methods

2.1. Chemicals and reagents

Curcumin from *Curcuma longa* (working standard) was obtained from Sigma-Aldrich (USA). The tested pharmaceutical formulation is a commercially available capsule (Root extract, *Curcuma longa*). Acetonitrile (ACN) was analytical grade. It was purchased from Sigma-Aldrich (USA). Disodiumhydrogen phosphate (NaH_2PO_4, pharmaceutical grade), and phosphoric acid were supplied from Merck (Germany). Ultrapure water was obtained from Barnstead NanoPure Diamond System. Methanol (MeOH) was analytical grade. It was purchased from Merck (Germany).

2.2 Instrumentation and conditions for the chromatography

The HPLC-UV determination was performed on an Agilent 1100 series HPLC equipped with a UV detector. The sample was separated on a Supelco Discovery 5 µm C18 (4.6mm x 5.0µm) column. Mobile phase was ACN:20 mM phosphate buffer, pH 6.0 (40:60 v/v). The flow rate was 1.5 mL min^{-1}. The injection volume was 20 µL, and U.V. detection was performed at 262 nm. The area of the peak eluted at 6.13 minutes was splitted and measured to perform the analysis.

2.3. Standard solution preparation

ACN was used to prepare a standard stock solution of curcumin (1000 µg mL^{-1}). Standard working solutions (0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 µg mL^{-1}) were prepared by diluting the stock solution with mobile phase solution.

2.4. Preparation of the sample solution

The powder in a capsule containing 400 mg of Turmeric root extract (*Curcuma longa*) was measured and found 500 mg. 500 mg powder was transferred to a 100 mL volumetric flask, 50 mL MeOH added, vortexed and left in a water bath for 30 minutes. Then 50 mL of water was added. The solution was left in a water bath for 30 minutes. Mobile phase was added to dilute the sample solution.

3. Results and Discussion

3.1. Method optimization

The first step in developing an analytical method is to decide which technique will be used for the analysis. Since it is not known what the product containing turmeric root extract contains other than the extract-

ISSN: 2458 - 8806

ed component(s), it was decided to work with HPLC. It was studied with reverse-phase HPLC and an attempt was made to shorten the analysis time as much as possible by using a short column. Preconditions as mobile phase started with an isocratic separation containing 80% organic solvent, but in order to save the main peak from the components also seen in the standard and thought to be curcuminoids (the main ones are presented in Table 1), 40% organic solvent and 60% buffer (pH was 6.0 when 20 mM phosphate buffer was used.) was the optimum condition. Unfortunately, the c urcumin peak could not be separated from curcuminoids as desired, but the similar profile with the standard solution matched the content of the product containing Turmeric root extract, making the analysis possible. The validated method demonstrated selectivity, linearity, accuracy and precision.

3.2. Method validation

The selectivity, linearity, sensitivity, accuracy, precision and robustness of this method have been validated according to ICH guideline Q2(R2) [28].

3.3. Selectivity

The selectivity of the developed method was proven by comparing the chromatograms of the standard solution, capsule and mobile phase. The peaks before the curcumin peak in both standard and capsule-containing curcumin extract were assumed to the demethoxycurcumin and bis-demethoxycurcumin (Figure 1). Some optimization techniques were attempted to rescue the curcumin peak from potential demethoxycurcumin and bis-demethoxycurcumin peaks. However, any method we employed could not improve the resolution between the curcumin peak and the others but the method was linear for the peak obtained at 6.13th minutes which belongs to the main compound, curcumin. Therefore, the method was accepted to be selective for the target molecule since there was not any unexpected peak coming from matrix components in our experimental conditions. All the validation procedures was performed using the area of the peak eluted at 6.13th minutes.

3.4. Linearity

A calibration curve was created with peak area values versus concentration for curcumin standards. Standard solutions contain 0.5, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 µg mL^{-1} of. Curcumin was prepared and injected into the HPLC system. The assessment of linearity was by means of linear regression analysis. The data for the linearity of curcumin is given in Table 2.

3.5. Sensitivity

The limit of the Detection (LOD) is the lowest amount of an analyte detectable in a sample. The limit of quantification (LOQ) is the lowest amount of an analyte for which quantitative analyses can be performed. The signal to noise ratio is used to estimate LOD and LOQ. The LOD is the value at three times the peak height of the baseline noise, while the LOQ

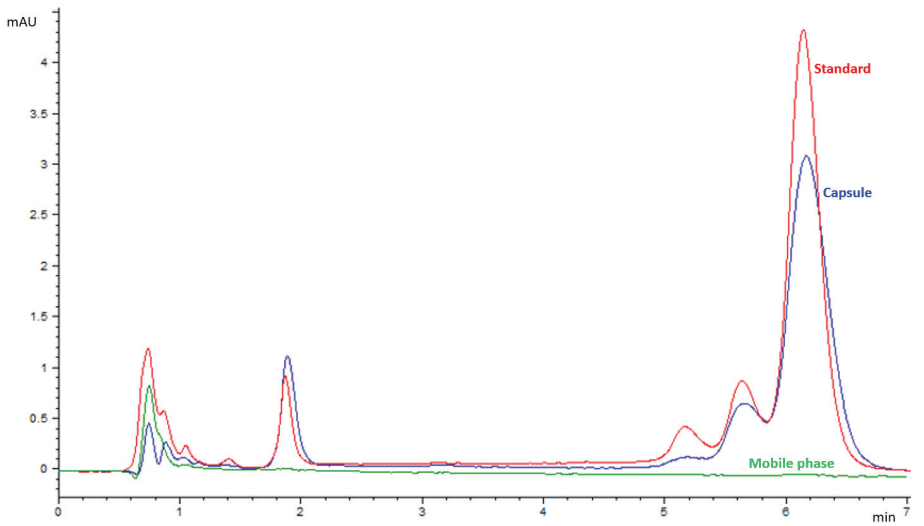


Figure 1. Chromatogram of standard solution, capsule and the mobile phase (Curcumin concentration is 5.0 µg mL-1 for the standard and capsule injections).

is the value at 10 times the peak height. The LOD of the HPLC method was determined to be 0.03 µg mL^{-1} and the LOQ was determined to be 0.10 µg mL^{-1} (Table 2).

3.6. Accuracy and Precision

The accuracy and precision of the test method was determined for intra-day and inter-day variations by analysing solutions six times. Standard curcumin solutions at three different concentrations (1.0, 5.0 and 20.0 µg mL^{-1}) were analysed on consecutive days (intra-day) and the same day (inter-day). The % relative standard deviation (RSD) was calculated for the precision of the method and the Bias% was calculated for the accuracy of the method (Table 3). As it is shown in Table 3, the method was precisely based on RSD% values, and the maximum RSD% value that we obtained was lower than 2.0. The method was accurate based on Bias% values, and the maximum Bias% value that we obtained was lower than 2.0 (Table 3). According to these results, the method that has been developed is precise and accurate.

3.7. Robustness

Analytical method robustness is the degree to which it is unaffected by minor changes in method parameters. Flow rate and wavelength were changed. At the 95% confidence interval, variations in wavelength had no significant effect on the response. On the other hand, the shift on the flow rate dramatically affected the result.

3.8. Analysis of the Capsules

The developed method was successfully applied to determine the curcumin amount in the commercial capsules containing curcumin extracts. The results are given in Table 5. In order to confirm the recovery values found lower than 90%, we applied a standard addition technique. Based on standard addition technique results, the calibration curve's slope was correlated with the one obtained for standard addition cure. Therefore, the results could confirm that the commercial capsule contains less than 90% of the claimed label.

4. Conclusions

A simple, accurate, selective and sensitive HPLC method was developed for the determination of curcumin in pharmaceutical analysis. The method was validated to demonstrate selectivity, linearity, accuracy, precision (inter- and intra-day) and sensitivity according to ICH guidelines. Although curcumin derivatives were also seen in HPLC separation with curcumin standard, and they failed to separate from the curcumin peak in method optimization studies, as seen in Figure 1, there wasn't interference from matrix components that could affect curcumin analysis. According to this chromatogram, the method is selective. A linear calibration curve was produced by regression analysis. Table 2 shows the results of the intra- and inter-day precision and accuracy studies. Both RSD% and Deviation were less than 2.0%, which proved that the developed method is precise and accurate. Wavelength change results were not significantly different ($p>0.05$).

Table 2. Linearity of curcumin analyses by the developed method (n=6).

Regression equation*	y = 16.773x - 0.6415
Linear range (µg mL^{-1})	0.5 - 50
R^2	0.9999
r	0.9999
Sa	0.1751
Sb	0.0531
LOD	0.03
LOQ	0.10

*y=bx+a, y: peak area, x: concentration, a: intercept, b:slope; R^2: Regression coefficient; r: correlation coefficient; Sa: Standard error of intercept and Sb: Standard error of slope; LOD: Limit of detection; LOQ: Limit of quantitation

ISSN: 2458 - 8806

Table 3. Precision and accuracy values of the developed methods for curcumin (n=6).

Added (µg mL^{-1})	Intraday			Interday		
	Found (µg mL^{-1})	Precision RSD%	Accuracy Bias%	Found (µg mL^{-1})	Precision RSD%	Accuracy Bias%
1.0	1.01 ± 0.0016	1.86	0.98	1.01 ± 0.0005	0.58	0.93
5.0	4.93 ± 0.0063	1.54	1.47	4.92 ± 0.0077	1.95	1.50
20.0	20.21 ± 0.0189	1.12	1.04	20.26 ± 0.0088	0.52	1.29

Found: mean ± standard error (n=6), RSD: Relative standard deviation, Bias: (Found - Added)/Added)x100

Table 4. The results of the robustness study of the developed method (Standard curcumin concentration is 5.0 µg mL-1).

	Standard method	Changes of the flow rate	Changes of the wavelength
Flow rate	1.5 mL/min	1.4 mL/min	1.5 mL/min
Wavelength	262 nm	262 nm	260 nm
$\bar{x}$	4.87 ± 0.03	5.18 ± 0.01	4.19 ± 0.01
RSD%	1.33	0.40	0.46
SD	0.06	0.02	0.02
t-test (p-value)		6.00E-07	3.46E-01

$\bar{x}$ (calculated concentration of curcumin): mean ± standard error, RSD: Relative standard deviation, SD: Standard deviation.

Table 5. The results of the analysed capsules with curcumin (Standard curcumin concentration is 5 µg mL-1).

Capsule Solutions	Analysis of commercially Available Capsule	
	Curcumin µg mL^{-1}	Recovery for Label Claim (%)
1	4.38	87.66
2	4.38	87.56
3	4.39	87.83
4	4.39	87.76
5	4.39	87.76
6	4.40	87.98
Mean ± SE	4.39 ± 0.0027	
RSD%	0.1517	

SE: Standard error, RSD: Relative standard deviation.

Thus, we developed an analytical method to determine a model for commercial curcumin capsules, and the method was proposed for other turmeric extract-containing capsules. This method could be used by supplement quality control laboratories, private laboratories, and university researchers and has been validated and contributed to the literature.

Acknowledgements

This study was performed using Department of Analytical Chemistry own sources.

Conflict of Interest

The authors declare that the contents in this article have no conflict of interest.

Statement of Contribution of Researchers

D.E., M.C. and S.A.: Design and Concept; M.C. and S.A.: Supervision; D.E.: Data collection/Processing; D.E.: Analysis; D.E., MC and D.D.: Writing; D.E., D.D. and S.A.: Critical review.

References

1. Zhou H, Beevers CS and Huang S. The targets of curcumin. Curr Drug Targets. 2011;12(3):332-347. https://doi.org/10.2174/138945011794815356
2. Shishodia S, Sethi G and Aggarwal BB. Curcumin: getting back to the roots. Ann N Y Acad Sci. 2005;1056(1):206-217. https://doi.org/10.1196/annals.1352.010
3. Indira Priyadarsini K. Chemical and structural features influencing the biological activity of curcumin. Curr Pharm Des. 2013;19(11):2093-2100. https://doi.org/10.2174/138161213805289228
4. Slika L and Patra D. A short review on chemical properties, stability and nano-technological advances for curcumin delivery. Expert Opin Drug Deliv. 2020;17(1):61-75. https://doi.org/10.1080/17425247.2020.1702644
5. Pari L, Tewas D and Eckel J. Role of curcumin in health and disease. Arch Physiol Biochem. 2008;114(2):127-149. https://doi.org/10.1080/13813450802033958
6. Pulido-Moran M, et al. Curcumin and health. Molecules. 2016;21(3):264. https://doi.org/10.3390/molecules21030264
7. Wakte PS, et al. Optimisation of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from *Curcuma longa*. Sep Purif Technol. 2011;79(1):50-55. https://doi.org/10.1016/j.seppur.2011.03.010
8. Banu KS and Cathrine L. General techniques involved in phytochemical analysis. IJARCS. 2015;2(4):25-32.
9. Yadav R and Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011;3(12).
10. Singh S and Aggarwal BB. Activation of transcription factor NF-κB is suppressed by curcumin (diferuloylmethane) (∗). J Biol Chem. 1995;270(42):24995-25000. https://doi.org/10.1074/jbc.270.42.24995.
11. Liang G, et al. Inhibition of LPS-induced production of inflammatory factors in the macrophages by mono-carbonyl analogues of curcumin. J Cell Mol Med. 2009;13(9b):3370-3379. https://doi.org/10.1111/j.1582-4934.2009.00711.x
12. Chen A and Xu J. Activation of PPARγ by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR. Am J Physiol Gastrointest and Liver Physiol. 2005;288(3):G447-G456. https://doi.org/10.1152/ajpgi.00209.2004
13. Durgaprasad S, Pai CG and Alvres JF. A pilot study of the antioxidant effect of curcumin in tropical pancreatitis. Indian J Med Res. 2005;122(4):315.
14. Hanai H, et al. Curcumin maintenance therapy for ulcerative colitis: randomised, multicenter, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol. 2006;4(12):1502-1506. https://doi.org/10.1016/j.cgh.2006.08.008
15. Phan TT, et al. Protective effects of curcumin against oxidative damage on skin cells in vitro: its implication for wound healing. J Trauma. 2001;51(5):927-931. https://doi.org/10.1097/00005373-200111000-00017
16. Aggarwal BB, et al. Curcumin: the Indian solid gold. Adv Exp Med Biol. 2007;595:1-75. https://doi.org/10.1007/978-0-387-46401-5_1
17. Hatcher H, et al. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci. 2008, 65(11):1631-1652. https://doi.org/10.1007/s00018-008-7452-4
18. Jagetia GC and Aggarwal BB. "Spicing up" of the immune system by curcumin. J Clin Immunol. 2007, 27(1):19-35. https://doi.org/10.1007/s10875-006-9066-7
19. Anand P, et al. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4(6):807-818. https://doi.org/10.1021/mp700113r
20. Kocaadam B and Şanlier N. Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health. Crit Rev Dood Sci and Nut. 2017;57(13):2889-2895. https://doi.org/10.1080/10408398.2015.1077195
21. Sri SN, et al. Curcumin-based food supplements: Challenges and future prospects. Nonvitamin and Nonmineral Nutritional Supplements. 2019;119-128. https://doi.org/10.1016/B978-0-12-812491-8.00016-3
22. Goel A, Kunnumakkara AB and Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. Biochem Pharmacol. 2008;75(4):787-809. https://doi.org/10.1016/j.bcp.2007.08.016
23. Maughan RJ. Quality assurance issues in the use of dietary supplements, with special reference to protein supplements.

ISSN: 2458 - 8806

J Nutr. 2013;143(11):1843S-1847S. https://doi.org/10.3945/jn.113.176651

24. Sanzini E, et al. Quality control of plant food supplements. Food Funct. 2011;2(12):740-746. https://doi.org/10.1039/c1fo10112a
25. Jayaprakasha GK, Jagan Mohan Rao L and Sakariah KK. Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. J Agric Food Chem. 2002;50(13):3668-3672. https://doi.org/10.1021/jf025506a
26. Jadhav BK, Mahadik KR and Paradkar AR. Development and validation of improved reversed phase-HPLC method for simultaneous determination of curcumin, demethoxycurcumin and bis-demethoxycurcumin. Chromatographia. 2007;65:483-488. https://doi.org/10.1365/s10337-006-0164-8
27. Kadam PV, et al. Standardisation and quantification of curcumin from *Curcuma longa* extract using U.V. visible spectroscopy and HPLC. J Pharmacogn Phytochem. 2018;7(5):1913-1918.
28. Guideline IHT. Validation of analytical procedures Q2 (R1). in International conference of harmonisation. Geneva, Switzerland. 2022.