International Journal of Medical and Pharmaceutical Sciences
Open access peer-reviewed Journal
Volume 2, Issue 8, Page 327-334.
https://doi.org/10.5281/zenodo.21833233

Research Article

Method Development and Validation of Curcumin, Piperine and Boswellic Acid in Polyherbal Formulation by RP-HPLC

Deepa Mokle*, Sachin Hiradeve, Namrata Mane, Lalchand Devhare, Vaibhav Uplanchiwar, Pravin Suruse, Vinod Thakre

Nagpur College of Pharmacy, Hingna, Nagpur-441110, Maharashtra, India

Background: Due to their anti-inflammatory and antioxidant characteristics, the use of polyherbal combinations including curcumin, piperine, and boswellic acid is very common. Standardization of these compounds relies on reliable analytical methods. Objective: The aim of this research is to develop and validate an efficient, accurate and precise RP-HPLC method for the determination of piperine, boswellic acid, and curcumin in a polyherbal combination. Method: The method of separation through the use of a mobile phase that is a mixture of acetonitrile and phosphate buffer was achieved using isocratic elution on a C18 column. The method was then validated based on linearity, accuracy, precision, robustness, LOQ, and LOD. Results: In addition to having very well-defined peaks, the method enabled a successful separation of curcumin, piperine, and boswellic acid. Linearity of the method was confirmed through calibration curves with correlation coefficients greater than 0.999. Although the precision of the method had % RSD values of less than 2%, its accuracy was proven through recovery experiments. Conclusion: To test the efficiency of quality control of polyherbal formulation containing curcumin, piperine, and boswellic acid, the validated RP-HPLC method is simple, accurate, and precise.

Keywords: RP-HPLC, Curcumin, Piperine, Boswellic Acid, Method Validation, Polyherbal Formulation.

INTRODUCTION

Highly sophisticated methods of separation are utilized to quantify and qualitatively analyze the components present in medicinal herbs or herbal preparations Curcumin, which is the major curcuminoid of Curcuma longa, shows antinflammatory, antioxidant, antimicrobial, and anticancer activities (Hewlings et al, Aggarwal BB et al). Piperine, which is an alkaloid in Piper nigrum, enhances the bioavailability of curcumin and acts as an antioxidant and anti-inflammatory agent (Srinivasan K et al, Shoba et al). The pentacyclic triterpenoids of Boswellia serrata resin are known as boswellic acids and are potent anti-inflammatory and anti-arthritic agents (Ammon HP et al, Siddiqui MZ et al). Out of all the constituents, 3-O-acetyl-11-keto-β-boswellic acid (AKBA) is the major one with pharmacological activity [6,7] (Ammon HP et al, Poeckel et al). It is very important to develop and validate methods for curcumin, piperine, and boswellic acid to control the quality of polyherbal preparations. The RP-HPLC method was developed in this study for the determination of curcumin, gallic acid, and piperine in the Turminice tablet. The Turminice tablet is employed for the treatment of osteoarthritis, rheumatoid arthritis, joint pain, and other inflammatory diseases. Curcumin, the bioactive component of Haldi, has anti-inflammatory, antibacterial, anti-allergic, and anti-cancer properties (Chainani Wu N. et al). The mode of action of curcumin as an anti-inflammatory agent can be explained by the binding of curcumin to its receptor; the activation of signaling pathway due to ligand-

receptor binding results in increased expression of PPAR-γ and thus inhibition of cytokine production (Jacob A et al). Piperine, which is one of the active ingredients of Piper, has the following activities such as nervous system depressant activity, anti-inflammatory, antipyretic, analgesic, antioxidant, and bioenhancer (Jain V et al, Kaouriya KG et al, Shah U et al). Piperine facilitates the intestinal absorption of different compounds and decreases gut metabolism of the drugs. Due to these actions, Piper finds use in many Ayurvedic preparations. Moreover, piperine improves the bioavailability of curcumin and gallic acid by inhibition of cytochrome P450 enzymes (Amar S et al). Boswellic acids are one of the primary biological pentacyclic triterpenes that occur in the gum of Boswellia serrata. There are several types of boswellic acids, among which β-boswellic acid (BBA), acetyl-β-boswellic acid (ABBA), 11-keto-β-boswellic acid (KBA), and 3-O-acetyl-11-keto-β-boswellic acid (AKBA) are predominant. AKBA is the most active ingredient Curcumin, piperine, and boswellic acid have strong anti-inflammatory, antioxidant, and anti-arthritic effects that help to increase the efficiency of Turminice Tablet based on a literature review. Piperine can increase the bioavailability of curcumin and show pharmacological activity. Boswellic acid is known for immunomodulatory and anti-inflammatory activity. Curcumin serves as a strong antioxidant and anti-inflammatory compound. As a result, the development of RP-HPLC method for the quality control of Turminice Tablet was achieved because curcumin, piperine, and boswellic acid were chosen as marker compounds. As per our knowledge, few studies have been conducted for simultaneous development and validation of curcumin, piperine, and boswellic acid in Turminice Tablet by RP-HPLC. Hence, an attempt has been made in the present study to develop a simple, accurate, precise, and robust RP-HPLC method for the simultaneous determination of these markers. The method has been validated as per ICH guideline Q2(R1) regarding linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ).

MATERIALS AND METHODS:

Instrumentation

Separation by chromatography was done by using Agilent HPLC Gradient System with auto sampler running ChemStation software. The separation was done using Agilent C18 column (250 mm × 4.6 mm, 5 µm). Spectrophotometric determination was done by using UV-Visible spectrophotometer (Analytical Technologies). The pH of mobile phase was determined using VSI pH meter (VSI 1-B). Weighing of standards and samples was done by using WENSAR™ High Resolution Balance. Sonication was done by using Ultrasonics electronic sonicator.

Standard Reagents

Piperine and curcumin reference standards were procured from Yarrow Chem Products, India. The reference standard of Boswellic acid was purchased from Konark Herbal and Healthcare Pvt Ltd, India. Acetonitrile, methanol, water, and other analytical grade solvents used in this study were purchased from reputed chemical suppliers.

Marketed Formulation

In order to design and validate the approach, Nexgen Healthcare Pvt. Ltd.'s Turminice pill was purchased from the local market. After then, the drug sample was stored properly until it was needed again.

Preparation of Standard Stock Solution

A 100 mL volumetric flask was prepared containing the accurately weighted amount of 300 mg of curcumin, 125 mg of boswellic acid, and 5 mg of piperine. For making the stock solution (Stock-I), the standards were solubilized in methanol with the help of sonication, and the volume was made up to the mark by adding methanol. The final concentrations of the stock solution were 25 µg/mL of piperine, 1250 µg/mL of boswellic acid, and 3000 µg/mL of curcumin.

Preparation of Working Standard Solutions

The stock solution was used to make appropriate dilutions with the mobile phase in order to get working solutions of desired concentrations for constructing a calibration curve and validation of the method. The validation parameters such as linearity,

accuracy, precision, etc. were determined by these solutions.

Preparation of Working Standard Solutions

Preparation of Sample Solution

The stock solution was used to make appropriate dilutions with the mobile phase in order to get working solutions of desired concentrations for constructing a calibration curve and validation of the method. The validation parameters such as linearity, accuracy, precision, etc. were determined by these solutions.

RP-HPLC method development

Selection of wavelength

The stock solution was used to make appropriate dilutions with the mobile phase in order to get working solutions of desired concentrations for constructing a calibration curve and validation of the method. The validation parameters such as linearity, accuracy, precision, etc. were determined by these solutions.

Chromatographic Conditions

The components curcumin, piperine, and boswellic acid were purified by chromatography through reverse-phase HPLC using a C18 column. Acetonitrile formed the optimum mobile phase: methanol:water mixture in the most suitable proportion (adjusted to pH 3.0 using orthophosphoric acid). Prior to running the sample, the mobile phase was degassed and filtered using a 0.45 µm membrane filter. The analysis was performed at an injection volume of 20 µL with a flow rate of 1.0 mL/min in isocratic mode. For detection, a UV detector was employed at the optimal wavelength while the temperature of the column was maintained at 25°C. Retention times for curcumin, piperine, and boswellic acid were identified to be 2.237 min, 3.890 min, and 5.982 min, respectively, under optimal chromatographic conditions. Optimal peak separation with satisfactory peak symmetry and system suitability criteria was achieved. All the three markers were simultaneously quantified from the polyherbal tablet formulation in the developed RP-HPLC method.

Method validation

Validation of the method was carried out according to the ICH Q2 (R1) Guidelines [15] for metrics such as specificity, linearity, precision, system precision, accuracy, detection limit, quantification limit, and robustness.

Specificity

Specificity refers to the ability to assess the analyte in the presence of components that would otherwise have been expected to be there. This is done to ensure that the marker component is detected, purified, and quantified from the Ayurvedic preparation that is being analyzed. Specificity is determined by comparing the component of interest separated from the commercial preparation with the retention time and the UV spectrum of standards.

Linearity

The linearity of the new RP-HPLC method was determined through a correlation between peak area and concentration for curcumin, piperine, and boswellic acid. Curcumin concentrations were varied from 30–150 µg/mL; piperine concentrations were from 0.25–1.25 µg/mL; and boswellic acid concentrations were from 12.5–62.5 µg/mL. All concentration levels were prepared and analyzed thrice. A plot of the average peak area as a function of the concentration of each compound facilitated the preparation of calibration curves. Good linearity was demonstrated by the regression analysis performed at the selected concentrations. The compounds curcumin, piperine, and boswellic acid demonstrated very good linear correlations between the concentration and the peak areas, with r^2 values of 0.9990, 0.9990, and 0.9993, respectively. This confirms the linearity and suitability of the developed RP-HPLC method for the quantitative determination of curcumin, piperine, and boswellic acid in the polyherbal product.

Precision

The accuracy of the developed methodology was ensured through testing the repeatability (intra-day accuracy) and inter-day accuracy, which showed a percent relative standard deviation (% RSD) below 2%. The results were presented in the table below table 5.

Accuracy

The recovery of the spiked standard in the medicine product was evaluated to determine the precision of the procedure. The results indicated that the procedure was precise, having an RSD% below 2 and a recovery range of 98-100.6%. A table outlined the results below in table 4.

Robustness

The difference in flow rate that showed no change occurred in the validation column and that showed the validation parameters such as theoretical plates, tailing factor, retention time, resolution, and percentage relative standard deviation of the peak were used to measure the robustness of the method. More than 2000 theoretical plates were obtained using the developed method, and the validity of the method was shown from the percentage relative standard deviation for the peak area and retention times that were less than 2 and tailing factor that was less than 2.

LOD and LOQ

LOD and LOQ were calculated using the slope of the calibration curve of curcumin and piperine and the standard deviation of the responses. This method is highly sensitive and can detect and quantify these compounds at lower concentrations based on the LOD and LOQ of 1.413 µg/ml and 0.007 µg/ml for curcumin and 1.973 µg/ml and 0.022 µg/ml for piperine and boswellic acid.

RESULTS:

Table 1: Linearity and range of curcumin

S. No.	Concentration(µg/ml)	Peak area
1	30	6981.60
2	60	15392.20
3	90	23601.15
4	120	31526.65
5	150	39644.60
Slope		271.3
Y-intercept		1008
Correlation coefficient		0.999

Table 2: Linearity and range of piperine

S. No.	Concentration(µg/ml)	Peak area
1	0.25	164.31
2	0.5	374.25
3	0.75	566.35
4	1	777.55
5	1.25	993.76
Slope		824.44
Y-intercept		43.41
Correlation coefficient		0.999

Table 3: Linearity and range of boswellic acid

S. No.	Concentration(µg/ml)	Peak area
1	12.5	1228.73
2	25	2550.70
3	37.5	3737.32
4	50	5006.97

5	62.5	6368.34
Slope		101.88
Y-intercept		42.23
Correlation coefficient		0.999

Table 4: Result for accuracy studies of Boswellic acid, curcumin and piperine

Selected Markers	Level of Accuracy	Theoretical content of marker (µg/ml)	Amount of marker added (µg/ml)	Total Amount of marker (µg/ml)	Amount of marker recovered (µg/ml)	% recovery	Mean recovery	%RSD
Piperine	80 100 120	0.25 0.25 0.25	0.2 0.25 3	0.45 0.50 0.55	0.20 0.25 0.30	99.69 0.49 0.55	99.07	0.89
Boswellic Acid	80 100 120	12.5 12.5 12.5	10 12.5 15	22.54 24.97 27.43	10.04 12.47 14.93	100.5 100.4 100.0	100.45	0.06
Curcumin	80 100 120	30 30 30	24 30 36	53.91 60.26 66.36	23.91 30.26 36.36	100.1 100.2 100.9	99.90	0.39

Table 5: Result for system precision- Interday

Level of conc.	Piperine			Boswellic acid			Curcumin		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
Avgarea of 3 d (ppm) n=3	373.52	569.16	821.1	2490.28	3741.82	5004.69	15397.95	23525.00	31497.55
SD	1.66	0.81	0.65	11.98	28.90	3.54	1.91	31.11	431.41
%RSD	0.44 %	0.14 %	0.08 %	0.48 %	0.77 %	0.07 %	0.01 %	0.13 %	1.37 %

Table 6: Result for system precision- Intraday

Level of con	Piperine			Boswellic acid			Curcumin		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
Avg. area of 3 d (ppm) n=3	368.83	566.68	777.72	2487.67	3770.90	5024.48	373.52	569.16	773.66
SD	5.01	3.68	2.17	10.83	5.59	63.20	17.82	145.73	575.37
%RSD	1.36 %	0.65 %	0.28 %	0.44 %	0.15 %	1.26 %	0.12 %	0.62 %	1.83 %

*conc = concentration, SD = standard deviation, mean for three independent analyses, %RSD = relative standard deviation, n= No. of injections.

Table 7: Result for LOD and LOQ values calculated from the calibration curve

Parameters	Piperine	Boswellic acid	Curcumin
LOD	0.007	1.973	1.413
LOQ	0.022	5.784	4.283

Table 8: System suitability of the developed method

Sample	Retention time	Area	USP Tailing Factor	USP Theoretical plates
Curcumin	5.982	6141.08	0.87	5877
Piperine	3.890	1058.60	0.77	4483
Boswellic acid	2.237	112.55	0.83	4764

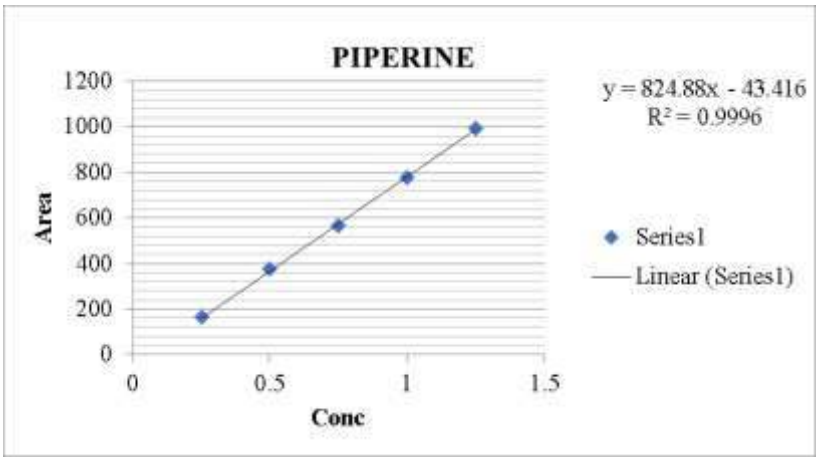

Fig.1. Calibration curve of piperine

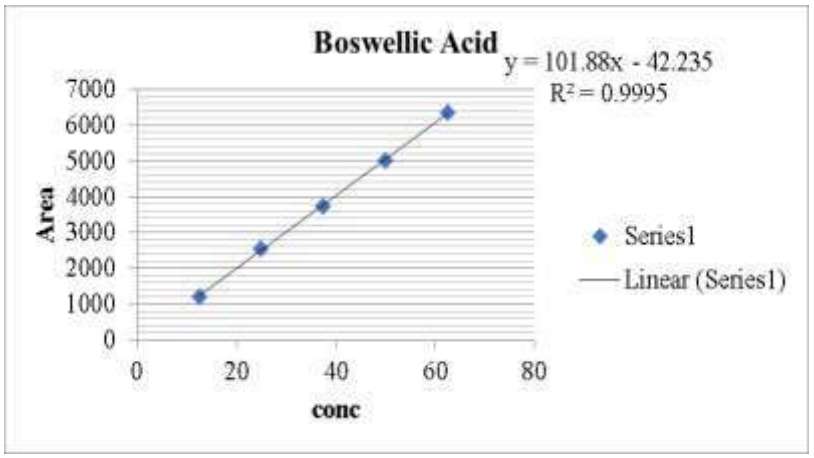

Fig.2. Calibration curve of Boswellic acid

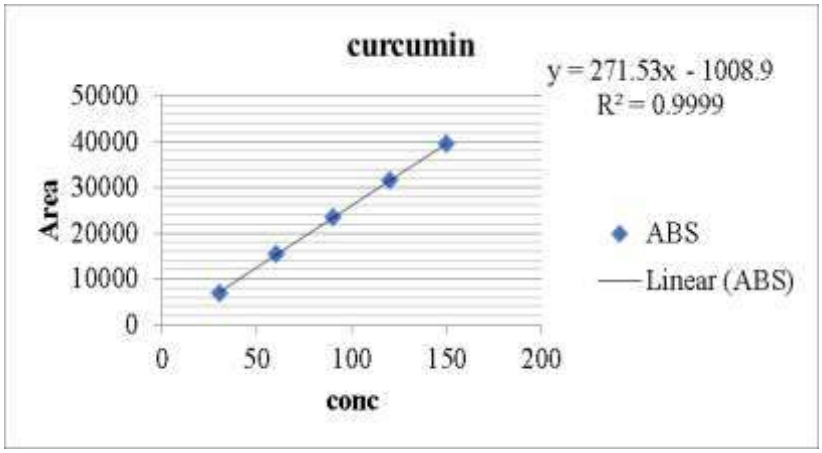

Fig.3. Calibration curve of Curcumin

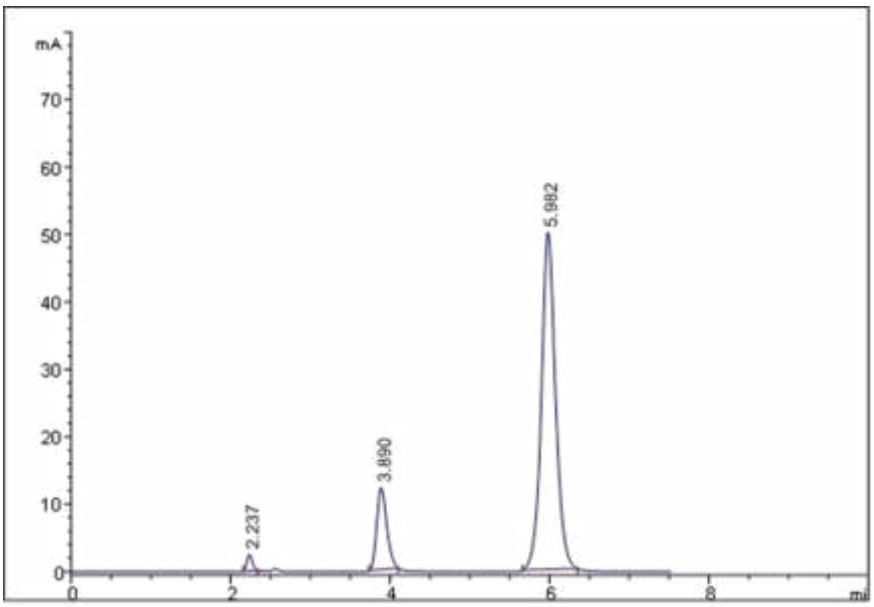

Fig. 4. Chromatogram of Standard of Curcumin Piperine and Bosswelic acid.

DISCUSSION:

A new, highly accurate, reliable, and precise RP-HPLC method has been developed and validated for simultaneous estimation of curcumin, piperine, and boswellic acid in a polyherbal tablet dosage form. In order to obtain optimum separation and resolution of peaks, various chromatographic parameters have been studied during method development. Acetonitrile was used as the mobile phase with a C18 column under optimum chromatographic conditions. Methanol: Water (isocratic elution with correction of pH by orthophosphoric acid). The analysis was performed using a column temperature of 25°C, injection volume of 20 µL, and flow rate of 1.0 mL/min. Detection was done using PDA detector at 244 nm. In view of the fact that the method did not encounter any interference from the formulating excipients in terms of the analyte retention times, the method exhibited specificity. As per the retention times of 2.237 minutes for curcumin, 3.890 minutes for piperine, and 5.982 minutes for boswellic acid, the peaks were distinctly separated. The results from the analysis of linearity within the selected ranges of curcumin (30-150 µg/mL), piperine (0.25-1.25 µg/mL), and boswellic acid (12.5-62.5 µg/mL) revealed the presence of a relationship between peak areas and concentrations of these compounds. For curcumin, piperine, and boswellic acid, the correlation coefficients (r^2) were 0. Experiments on LOD and LOQ have been done in order to find out the sensitivity of the method. LOD of curcumin, piperine, and boswellic acid has been found out to be 1.413 µg/mL, 4.283 µg/mL, and 5.784 µg/mL, respectively. This shows that the method is able to detect the analytes in low concentrations. Reproducibility and reliability of the method has been established by means of precision experiment, repeatability, intraday precision, and inter-day precision. These experiments provided us with %RSD in an acceptable limit of less than 2%. The accuracy of the developed method has been validated through accuracy experiments, which include recovery experiments. Retention times, better peak symmetry, remarkable resolution, higher sensitivity, and enhanced selectivity using PDA detection are some of the advantages that the new RP-HPLC method possesses in comparison with the earlier methodologies. The suitability of the method for quality control testing of curcumin, piperine, and boswellic acid in polyherbal tablet formulations was confirmed once it was validated as per ICH Q2(R1).

CONCLUSION

For the compounds such as curcumin, piperine, and boswellic acid in the polyherbal tablet formula, a simple, fast, precise, accurate, specific and robust RP-HPLC technique has been developed and validated. The three marker compounds were effectively separated under optimized chromatographic conditions, and the retention times of curcumin, piperine, and boswellic acid were 2.237, 3.890, and

5.982 minutes, respectively. The developed procedure was validated for specificity, linearity, accuracy, precision, robustness, LOD, and LOQ according to the ICH Q2(R1) guidelines. As the correlation values exceeded 0.999, high linearity was observed within the concentration range of 30-150 µg/mL for curcumin, 0.25-1.25 µg/mL for piperine, and 12.5-62.5 µg/mL for boswellic acid. Moreover, satisfactory sensitivity, accuracy, and precision were observed, and all the parameters of validation fulfilled the predefined acceptance criteria.

ACKNOWLEDGMENT:

The authors extend their gratitude to the Nagpur College of Pharmacy, Nagpur for giving an opportunity to carry out work in their college.

REFERENCES

1. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against various diseases. Int J Biochem Cell Biol. 2009;41(1):40-59.
2. Hewlings SJ, Kalman DS. Curcumin: A review of its effects on human health. Foods. 2017;6(10):92.
3. Srinivasan K. Black pepper and its pungent principle-piperine: A review of diverse physiological effects. Crit Rev Food Sci Nutr. 2007;47(8):735-48.
4. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. 1998;64(4):353-6.
5. Siddiqui MZ. Boswellia serrata, a potential anti-inflammatory agent: An overview. Indian J Pharm Sci. 2011;73(3):255-61.
6. Ammon HP. Boswellic acids in chronic inflammatory diseases. Planta Med. 2006;72(12):1100-16.
7. Poeckel D, Werz O. Boswellic acids: Biological actions and molecular targets. Curr Med Chem. 2006;13(28):3359-69.
8. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines—A review. Int J Biodivers Conserv. 2012;4(3):101-12.
9. Chainani Wu N. Safety and anti-inflammatory activity of curcumin: a component of turmeric (Curcuma longa). J Alternative Complementary Med 2003; 9:161-8.
10. Jacob A, Zhou Wu RM, Wang P. Mechanism of the anti-inflammatory effect of curcumin: PPAR-γ activation. PPAR Res 2008. Doi:10.1155/2007/89369.
11. Jain V, Shaikh SGM. Development and validation of a RP-HPLC method for the simultaneous determination of curcumin, piperine and camphor in an ayurvedic formulation. Int J Pharm Pharm Sci 2018; 10:115-21.
12. Kaouriya KG, Parmar PM, Topiya HR, Faldu SD. Method development and validation of rifampicin and piperine in their combined dosage form. Int Bull Drug Res 2012; 1:71-80.
13. Shah U, Jasani A. UV spectrophotometric and RP-HPLC methods for simultaneous estimation of isoniazid, rifampicin and piperine in pharmaceutical dosage. Int J Pharm Pharm Sci 2014; 6:274-80.
14. Amar S, Amar D. Piperine: a bio enhancer. Int J Pharm Res Tech 2011; 1:1-5.
15. International Conference on Hormonization of Technical Requirement for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedure: Text and Methodology ICH Q2(R1); 2005.

Cite: Deepa Mokle*, Sachin Hiradeve, Namrata Mane, Lalchand Devhare, Vaibhav Uplanchiwar, Pravin Suruse, Vinod Thakre, Method Development and Validation of Curcumin, Piperine and Boswellic Acid in Polyherbal Formulation by RP-HPLC, Int. J. Med. Pharm. Sci., 2026, 2 (8), 327-334. https://doi.org/10.5281/zenodo.21833233