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Novel Validated HPLC Method Development for Simultaneous Analysis of Curcumin and β-Boswellic Acid

Premanand A. Nighojkar[*1], Munira Momin[2], Vandana Jain[3]
1.Pacific Academy of Higher Education and Research University, Pacific hills, Pratap Nagar Ext., Udaipur, Rajasthan. 313 003
2.SVKM's Dr.Bhanuben Nanavati College of Pharmacy, Mithibai college campus, Vile Parle (W), Mumbai. 400 056
3. OES's Oriental College of Pharmacy, Sector 2, Sanpada, Navi Mumbai. 400 705

ABSTRACT

A simple, rapid and specific High performance liquid chromatography (HPLC) method has been developed for simultaneous analysis of Curcumin and β-Boswellic acid in a prepared polyherbal gel formulation containing turmeric and boswellia extracts. High performance liquid chromatography analysis was performed on a C_{18} column using 90:10 (v/v) mixture of acetonitrile and water as isocratic mobile phase at a flow rate of 1 ml/min. Ultra violet detection was set at 425 nm for Curcumin and 242 for β-Boswellic acid. The method was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with International Conference on Harmonization guidelines. Validation data reveals that the method is specific, accurate, precise, reliable and reproducible. Good linear correlation coefficients (r^2>0.9993) were obtained for calibration plots in the range tested. Limit of detection for Curcumin was 0.16 µg/ml and limit of quantification was 0.50 µg/ml while for β-Boswellic acid limit of detection (LOD) and limit of quantification (LOQ) were found to be 3.3 and 9.9 µg/ml respectively. Recovery was found to be between 98.75 to 99.01 % for Curcumin and from 98.72 to 100.01 % for β-Boswellic acid. The established HPLC method is appropriate and the two selected markers are well resolved, enabling efficient quantitative analysis of Curcumin and β-Boswellic acid. The method was successfully used for quantitative analysis of these two marker constituents in an in-house prepared polyherbal gel formulation.

Key word: HPLC, Simultaneous analysis, Polyherbal gel formulation, Curcumin, β-Boswellic acid

*Corresponding Author Email: nighojkar.5683@gmail.com
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INTRODUCTION

Turmeric (*Curcuma longa* L., Family: Zingiberaceae) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases[1]. Typical chemical constituents of the rhizomes are the yellow pigments (curcuminoids 2-8%) and an essential oil (4-8%). A typical essential oil contains over 25 compounds, mostly sesquiterpenoids and the major ones are zingiberene (~25%), turmerones (~ 30 %, both α and β-turmerone), and *ar*- turmerone (~25%) [2].Of the 10 or so curcuminoids, the main constituent (>90%) is curcumin (curcumin I). Closely related compounds (figure 1) are desmethoxycurcumin (curcumin II), bisdesmethoxycurcumin (curcumin III), and dihydrocurcumin. Biologically active polysaccharides ukonan A, B, C and D have also been isolated[3]. It is reported to possess anti-inflammatory, antirheumatic, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal and antiviral effect[4].

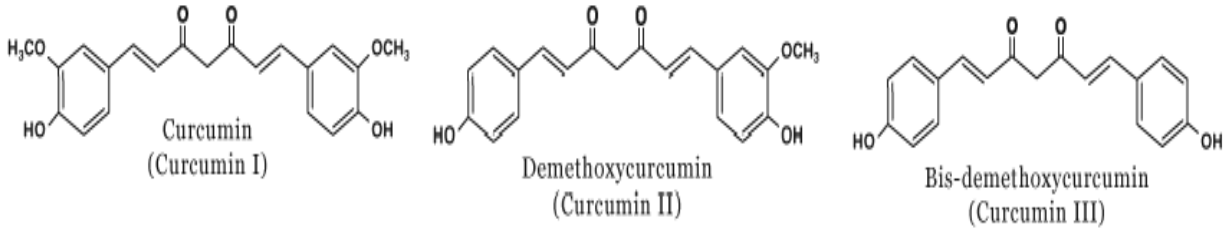

Figure 1: Chemical constituents of turmeric

Boswellia serrata possess oleo-gum-resin. The volatile oils contain alpha thujene and p-cymene. The resin (gugguls) contains a mixture of terpenoids made up of four pentacyclic triterpene acids (figure 2): β-boswellic acid (the most abundant), 3-O-acetyl β boswellic acid (ABA), 11-keto-β-boswellic acid, and 3-O-acetyl-11-keto-β-boswellic acid (AKBA), collectively called as boswellic acids. Standardized extracts from commercial sources usually contains 37.5-65% boswellic acid. The gum resin contains arabinose, galactose, xylose, galacturonic acid and digitoxose. The other constituents reported from boswellia are: D-fructose, D-lactose, D-glucose, L-sorbose, raffionse, raminose, and D-galactose[5]. It is used as anti-inflammatory, antirheumatic, antiseptic, in dysmenorrhea, amenorrhea, stomach pain, ulcers etc[6-7].

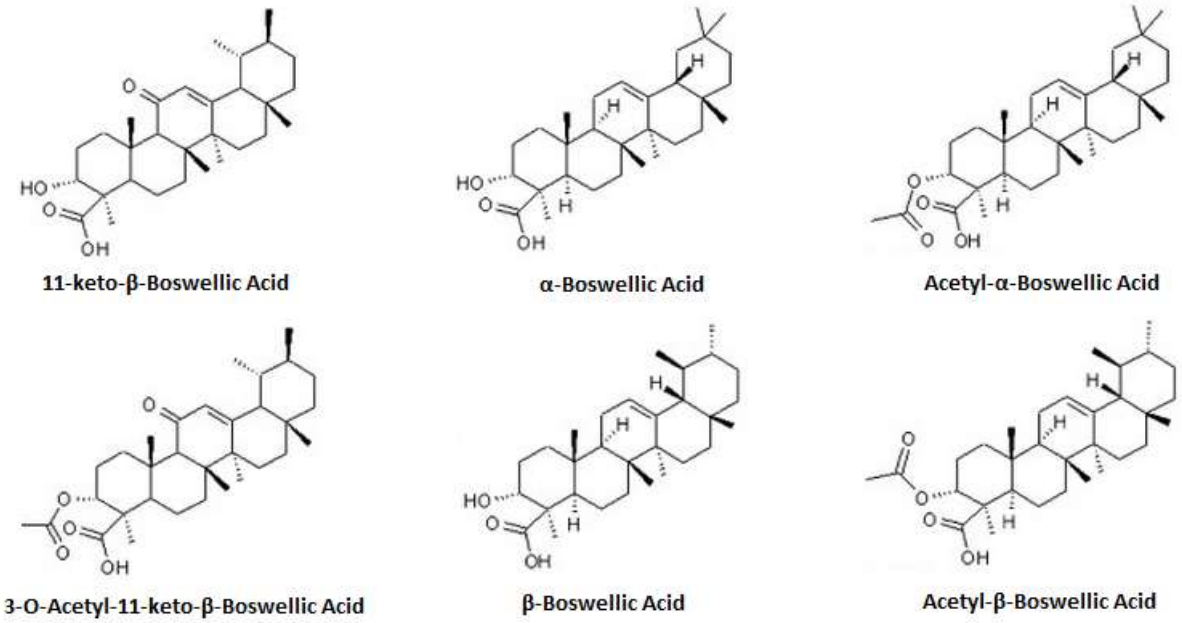

Figure 2: Chemical constituents of Boswellia

Variety of HPLC methods were reported for determination of Curcumin with different mobile phases, Methanol, Acetonitrile and Alcohol[8], Acetonotrile: Citric buffer pH 3.0[9], Acetonotrile 5%: Acetic acid[10], methanol: ammonium acetate[11]. Quantitative determination of Boswellic acid is also reported with different mobile phases like methanol: acetonitrile: water[12] and hexane: methanol: chloroform[13] .

MATERIALS AND METHOD

Plant Material:

Curcumin and β-Boswellic acid (purity 95≥ % by HPLC) was purchased from Sigma-Aldrich. HPLC grade solvents were used for HPLC analysis and were purchased from SD Fine (Mumbai, India).

Reagents and solutions:

Stock solution of curcumin containing 10µg/ml was prepared in methanol. Calibration standard solutions of six different concentrations 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 µg/ml were prepared by appropriate dilutions. Stock solution of β- Boswellic acid 1 mg/ml was prepared in methanol. Calibration standard solutions of concentrations 10, 20, 40, 80, 120 and 400 µg/ml were prepared by appropriate dilutions.

Sample Preparation:

Gel preparation: Nanostructured lipid carrier gel was formulated having 0.1% Curcumin and 0.3 % β-boswellic acid as active component, Labrasol as liquid lipid, Compritol HD5 ATO as solid lipid and Leciva S70 as surfactant with Carbopol 940 as gelling agent.

1 gram of prepared gel was dissolved in 100 ml of methanol. The stock solution was injected after suitable dilutions. The sample was analyzed by HPLC at 425 nm for initial 5 min followed by wavelength of 242 nm.

Chromatographic condition:

HPLC analysis was performed with Shimadzu, SPD-20 a system consisting of a pump 2LC-10AT with UV-Vis detector. Compounds were separated on 150 mm x 4.6 mm i.d. 5 µm Shim Pack VP-ODS, Phenomenex C18 column with 90:10 (v/v) Acetonitrile: Water as isocratic mobile phase at a flow rate of 1 ml/min. The injection volume was 20 µL and detection wavelength was 425 nm for curcumin followed by 242 nm for β- Boswellic acid. HPLC was performed at ambient temperature and data were analyzed on a computer equipped with LC Solution software.

Method validation:

The optimized chromatographic method was validated according to the procedure described in ICH guidelines Q2 (R1) for the validation of analytical methods.

Linearity:

The linearity of calibration curve in Curcumin and β-Boswellic acid standard solution over the concentration range of 0.5-5 μg/ml and 10 to 400 μg/ml respectively, through proposed HPLC method was carried out. Results were subjected to regression analysis.

Limit of detection and limit of quantification:

Limit of detection and limit of quantification were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations, LOD= 3.3* σ/slope and LOQ = 10* σ/slope, Where σ= standard deviation

Precision:

The precision of the method was determined by inter-day and intra-day reproducibility experiments of the proposed method. All the samples were analyzed in triplicate and mean was calculated.

Accuracy:

The accuracy of the proposed method was determined by recovery study, which was carried out by adding standard Curcumin and β-Boswellic acid in pre analyzed gel. The gel sample was spiked with three different amounts of standard Curcumin and β-Boswellic acid prior to extraction. The spiked samples were extracted in triplicate and analyzed under the previously established optimal conditions and percent recovery was calculated for both the marker compounds

RESULTS AND DISCUSSION

The method was optimized by varying the flow rate and the relative amounts of acetonitrile and water. Use of low concentration of acetonitrile (75%) and flow rate of 1 ml/min resulted in slow elution but with sharp chromatographic peaks. Increasing the amount of acetonitrile resulted in rapid and sharp peaks with better resolution. When 90:10 (v/v) acetonitrile-water was used at a flow rate of 1 ml/min, Curcumin and β-Boswellic acid were eluted at 3.32 and 8.44 min, respectively. The wavelengths of maximum of absorption (λ_{max}) of Curcumin and β-Boswellic acid were 425 and 242 nm respectively, hence the detector was programmed at 425 nm for initial 5 min for curcumin and then at 242 nm for β-Boswellic acid.

In the optimized chromatographic conditions a good separation of selected marker constituents was obtained in mixed standard solution (figure 3) with a retention time of 3.32 min for Curcumin and 8.44 min for β-Boswellic acid. Chromatographic peaks were identified by comparing their retention times under the same operating conditions. Spiking the sample solution with the standard compound was also used to assist confirmation of peak identity (figure 4).

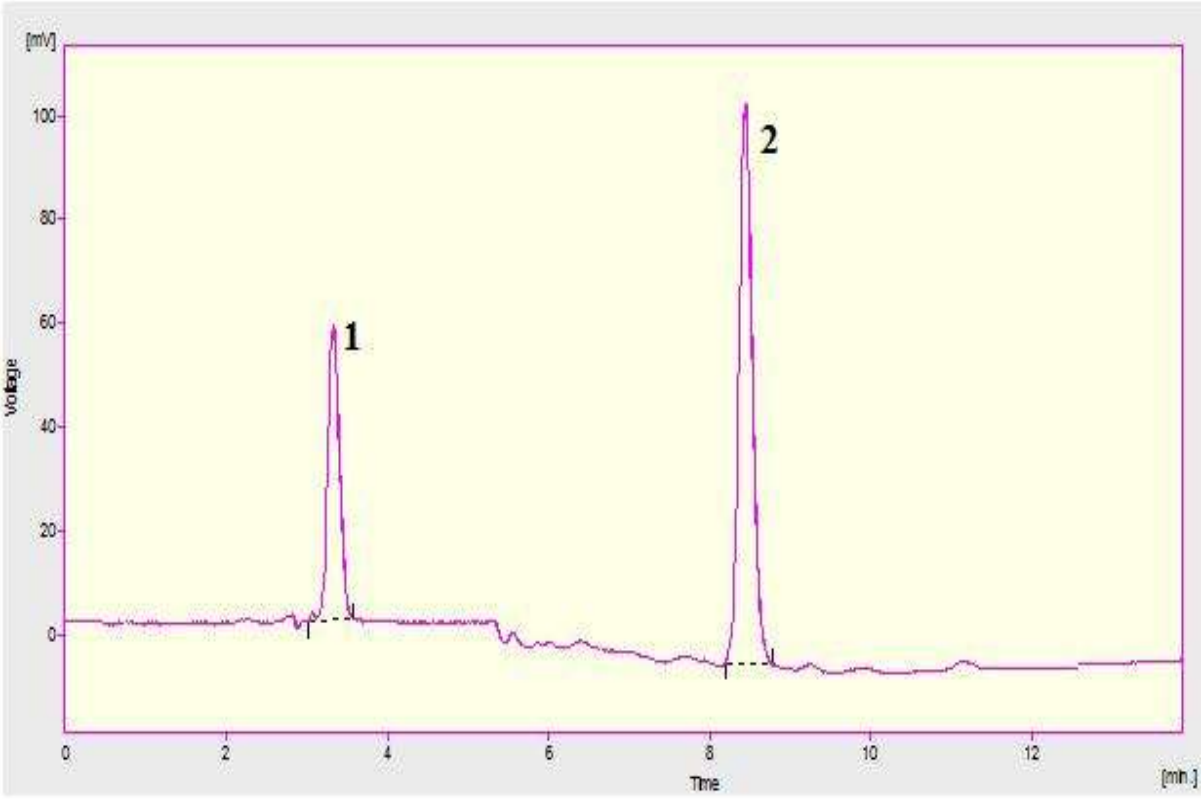

Figure 3: HPLC chromatogram of reference standard showing Curcumin (1) and β-Boswellic acid (2).

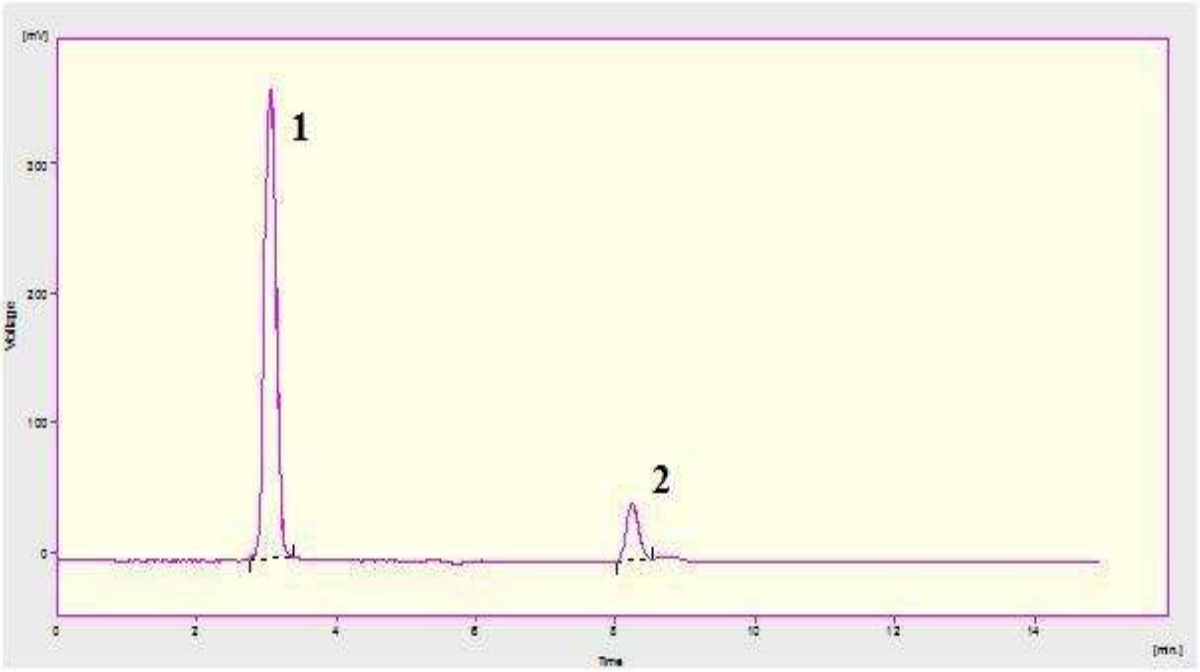

Figure 4: HPLC chromatograms of prepared polyherbal gel formulation showing Curcumin (1) and β-Boswellic acid (2).

The method was found to be linear in the range of 0.5- 5 µg/ml with a correlation coefficient of 0.9993 for curcumin (Table 1). The LOD and LOQ of curcumin were found to be 0.16 and 0.50 µg/ml respectively. The low % RSD values of for inter-day and intra-day variation reveals that the proposed method is precise (Table 2). The high recovery values (98.78-99.04) indicate a satisfactory accuracy (Table 3).

Table 1: Regression parameters, linearity, limit of detection and limit of quantification of Curcumin and β- Boswellic acid

Drug	Conc. Range (µg/ml)	RT(min)	Regression equation	R^2	LOD (µg/ml)	LOQ (µg/ml)
Curcumin	0.5-5	3.32	y = 104.71x + 9.6975	0.9993	0.165	0.501
β- Boswellic acid	10-400	8.44	y = 0.9093x - 1.3824	0.9999	3.301	9.995

Table 2: Precision of inter-day and intra-day HPLC measurement for Curcumin and β-Boswellic acid

Drug	Intra-day[b] Content[a] (%)	% RSD	Inter-day[c] Content[a] (%)	% RSD
Curcumin	0.995	1.05	0.994	1.17
β- Boswellic acid	0.306	1.0	0.309	1.22

[a]= Mean of three readings

[b]= Samples were analyzed three times a day

[c]= Samples were analyzed once a day over three consecutive day

Table 3: Recovery studies for Curcumin and β- Boswellic acid

Analyte	Contained (µg/ml)	Added (µg/ml)	Found (µg/ml)	Recovery (%)	Mean % recovery
Curcumin	9.95	0.5	10.32	98.75	98.91
		2	11.83	98.99	
		5	14.80	99.01	
β- Boswellic acid	30.6	10	40.18	98.96	99.23
		40	70.60	100.01	
		120	148.68	98.72	

For boswellic acid the method was found to be linear in the range of 10-400 µg/ml with a correlation coefficient of 0.9999 (Table 1). The LOD and LOQ of β-Boswellic acid were found to be 3.30 and 9.99 µg/ml respectively. The method was found to be précised with low % RSD values for inter-day and intra-day variation in boswellic acid (Table 2). The high recovery values (98.99-100.01) indicates a satisfactory accuracy (Table 3).

Therefore the present HPLC method can be regarded as accurate and precise for the simultaneous estimation of Curcumin and β-Boswellic acid in gel formulation.

These results revealed that the method enables rapid, sensitive and highly accurate simultaneous quantification of Curcumin and β-Boswellic acid. When the method was subsequently used for analysis of a prepared polyherbal gel formulation, the amount of Curcumin and β-Boswellic acid were found to be 0.995 % and 0.309 % respectively.

CONCLUSION

The present study indicates that this method enables rapid, sensitive, precise and highly accurate simultaneous quantification of Curcumin and β-Boswellic acid. It can be used for quality control and quantitative analysis of prepared polyherbal gel formulations containing turmeric and boswellia extracts using Curcumin and β-Boswellic acid as marker compound.

REFERENCE

1. Ammon HPT and Wahl MA. Pharmacology of *Curcuma longa*. Planta Med 1991; 57: 1-7.
2. Sukh Dev. Prime Ayurvedic Plant Drug. 2nd Edition. New Delhi. Ane books Pvt Ltd; 2012: 297-306.
3. Roth GN, Chandra A, Nair MG. J Nat Prod. Novel bioactivities of *Curcuma longa* constituents. 1998; 61(4): 542-545.
4. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee R. Turmeric and Curcumin: biological actions and medicinal applications. Current Science 2004; 87(1): 44-53.
5. Dawson S. Boswellia spp. Plant monograph. Herbal medicine. Class of Sept; 2006; 1-21.

6. Sultana A, Rahman K, Padmaja AR, Rahman S. *Boswellia serrata* Roxb. A traditional herb with versatile pharmacological activities: a review. IJPSR 2013; 4(6): 2106-2117.
7. Wu JN. Chinese Materia Medica. First edition. New York, USA. Oxford University press; 2005: 144-145.
8. Sakariah KK, Guddadarangavvanahally KJ, Rao LJM. Improved HPLC method for detection of curcumin, Demethoxycurcumin, and Bisdesmethoxycurcumin. J Agric Food Chem 2002; 50(13): 3668-3672.
9. Samual J, Zengshuan Ma, Shaveganpour A, Brocks DR, Lavasanifar A. High performance liquid chromatography analysis of curcumin in rat plasma: application to pharmacokinetics of polymeric micellar formulation of curcumin. Biomedical Chromatography 2007; 21(5): 546-552.
10. Li J, Jiang Y, Wen J, Fan G, Wu Y, Zhang C. A rapid and simple HPLC method for the determination of curcumin in rat plasma: assay development, validation and application to a pharmacokinetic study of curcumin liposome. Biomed Chromatogr 2009; 23(11): 1201-1207.
11. Memvanga PB, Mbinze JK, Rozet E, Hubert P, Preat V, Marini RD. Development of a liquid chromatographic method for the simultaneous quantification of curcumin, β-arteether, tetrahydrocurcumin and dihydroartemisinin, application to lipid based formulations. J Pharm Biomed Anal 2014; 88: 447-456.
12. Rajendra CE, Shashihara S, Hanumantharaju N, Nadaf MA, Mohan CG. HPLC estimation of Withaferin-A and Boswellic acid in formulated gels. Int J of Pharm Biological Archives 2010; 1(2): 183-186.
13. Shah SA, Rathod IS, Suhagia BN, Patel DA, Parmar VK, Shah BK, Vaishnavi VM. Estimation of boswellic acids from market formulations of *Boswellia serrata* extract and 11-keto beta-boswellic acid in human plasma by high performance thin layer chromatography. J chromatogr B Analyt Technol Biomed Life Sci 2007; 848(2): 232-238.