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RESEARCH ARTICLE

EVALUATION OF VARIOUS MARKETED FORMULATIONS OF BOSWELLIA BY RP-HPLC and HPTLC

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ABSTRACT

The quality of herbal products which varies with the time under the influence of environmental factors, such as temperature, humidity, moisture, other ingredient or excipient in the dosage form, microbial contamination, adulteration, lack of good manufacturing practices, poor quality control procedures, poor agriculture and post harvesting methods. Therefore qualitative and quantitative evaluation of herbal drugs can serve as an important tool for monitoring the safety herbal formulations. The present study was performed quantitative assay, method development and validation of Boswellic acids using HPLC and HPTLC and also its application in various marketed formulations of Boswellia. Method Validation was performed as per the ICH guidelines. From all the validation parameters it is very clear that the HPLC and HPTLC method developed was sensitive, accurate, precise, linear, rugged, robust, and convenient and it could be applicable for quality control studies of marketed formulations of Boswellia.

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INTRODUCTION

The demand for herbal products is growing day by day throughout the world and major pharmaceutical companies are currently conducting extensive research work on plant materials for their potential medicinal value. Many countries are registering their standardized herbal drugs with proven clinical efficacy, safety and as dietary supplements. But India is unable to exploit the world market due to unsatisfactory system of quality control which necessitates the establishment of standards for herbal single drugs and for compound formulations. The quality of herbal products which varies with the time under the influence of environmental factors, such as temperature, humidity, moisture, other ingredient or excipient in the dosage form, microbial contamination, adulteration, lack of good manufacturing practices, poor quality control procedures, poor agriculture and post harvesting methods. Arthritis is a group of painful and degenerative conditions marked by inflammation in the joints that causes stiffness and pain. Diet and exercise are essential parts of the treatment of this problem, but use of traditional medicine, derived from plants are the major form of treatment. Boswellia is world widely used as a anti inflammatory to treat arthritis (Pallavi *et al.,* 2014; Preeti and Aakash, 2015; Sunita and Ram, 2013; Sultana *et al.,* 2013). Therefore qualitative and quantitative evaluation of herbal drugs can serve as an important tool for monitoring the safety herbal formulations. In the present study, Boswellic acids content in different marketed formulations of Boswellia are determined by different analytical methods to assure their quality, safety and efficacy.

MATERIALS AND METHODS

Herbal Formulations Selected for the Study: The standard Boswellic acids were purches from Yucca Enterprises (Mumbai), and five herbal formulations selected for the study were procured from local market they are: Himalaya (karnataka), Morpheme-(Haryana), Gufic Bio sciences (Mumbai), Herbal Hills (Mumbai) and Health vit (Ahmedabad).

Chemicals and instuments: All the chemicals and reagents used in different processes were procured from Merk India and Sigma-Aldrich chemicals private limited-Mumbai. Instruments used were Shimadzu liquid chromatography system, an LC -10 AT model pump, and Diode Array Detector and HPTLC (M/s Anchrome and Camag Ltd.).

Quantitative evaluation of marketed formulations of Boswellia **by HPLC method**

HPLC Method: Shimadzu liquid chromatography system, an LC -10 AT model pump, and Diode Array Detector and Analysis was performed on a reverse phase C_{18} column (300 X

3.9 mm); particle size 10 µm. The composition of the mobile phase was optimized by trial and error. Mobile phase is optimized as using methanol: Acetonitrile: water: Galcial acetic acid: (90:0.5:9.5) at UV detection 274 nm with a flow rate of 1ml/min (Shailesh *et al.,* 2007).

Preparation of standard solutions: Standard solution (1mg/ml) of different concentrations (10-60 µg/ml) was prepared using millipore water. Different concentrations of the standards were injected into the HPLC by using the Mobile phase.

Preparation of sample solutions: Samples of different herbal formulations were finely powdered and weighed (1 gm each) and extracted with water. Extract diluted up to 100 ml with water. Samples were filtered through millipore filter (0.2 µm) and a known amount of each extract was injected to HPLC analysis. The content of Boswellic acid present in extracts was calculated using calibration graph of each compound.

Percentage of Assay (HPLC): According to Indian Pharmacopoeia (IP), the test is not valid unless the relative standard deviation (RSD) for the replicate injections is not more than 2.0 percent. Therefore the selected powders, tablets and capsule have passed the test as RSD of replicate injections was found to be less than 2% given in the (Table 1) when injected into HPLC.

Quantitative evaluation of marketed formulations of Boswellia by HPTLC METHOD

HPTLC Method: The HPTLC system consisted of a CAMAG Linomat IV-automatic spotting device, a CAMAG twin-trough glass chamber (20 X 10cm), a CAMAG TLC scanner-3, CAMAG WinCATS-4 software version, and a 100ul HPTLC syringe (SGE, Australia).

Chromatographic Parameters: Chromatography was performed on silica gel $60F_{254}$ HPTLC layers (10 x 10 cm; 0.3 mm layer thickness).Samples and standard compounds of known concentrations were applied as 6 mm wide bands using automated TLC applicator with nitrogen flow providing delivery speed 150 ŋl/s from the syringe. These parameters were kept constant throughout the analysis (Shirwaikar *et al.,* 2005).

Preparation of Standard Solutions: Standard solutions of Boswellic acid (1 mg/5 ml) were prepared using millipore water. Standard graph was plotted in the concentration of 200-1000 ng/ml.

Extraction of Samples: Each 4 gm of different herbal branded formulation was sonicated with 70% methanol (3 x 20 ml) for about 45 min. Then the extract was filtered in a Buchner funnel using Whatman No. 1 filter paper and was concentrated under vacuum in a rotary evaporator at 50°C, redissolved in methanol and finally reconstituted in 20 ml methanol prior to HPTLC analysis (Shailesh *et al.,* 2007; Shirwaikar *et al.,* 2005; Vivek *et al.,* 2016; Reich *et al.,* 2007).

Detection and Quantification of Boswellic acid: After completion of sample application, the plate was developed in a Camag Twin through glass tank presaturated with mobile phase of Hexane: Ethyl acetate (7 : 3) for one hour. The TLC was performed under laboratory conditions of 25 ± 2°C and 60% relative humidity. After development the plates were taken off and dried. Boswellic acid was quantified using a Camag TLC Scanner model 3 equipped with Camag Wincats software applying the following conditions: slit width 6 x 0.45 mm**,** wavelength (λmax) 350 nm**,** and absorption reflection scan mode. The identification of Boswellic acid in formulations was confirmed by superimposing the UV spectra of samples and standards within the same R_f window (Reshma *et al.,* 2013; Pawar *et al.,* 2011; Shailesh *et al.,* 2008; Shirwaikar *et al.,* 2005).

Percentage of Assay of Boswellic acids (HPTLC): According to Indian Pharmacopoeia (IP), the test is not valid unless the relative standard deviation (RSD) for the replicate injections is not more than 2%.Therefore the selected powders, tablets and capsule have passed the test as RSD of replicate injections was found to be less than 2% as depicted in the (Table 1) by HPTLC.

Validation Method for HPLC and HPTLC

The methods for HPLC and HPTLC validation according to ICH guidelines are depicted below:

Linearity and Range: The linearity of the method was evaluated by analyzing a series of standard solutions. Five different concentrations of standard solutions in the range of 10-60 and 10-60 mg/ml were tested in HPLC and HPTLC respectively, and each was injected in triplicate. After both chromatograms value obtained, a standard calibration curve was plotting by using the concentration of standard solutions versus peak area and correlation coefficient and regression line equations were determined (Vivek *et al.,* 2016).

Specificity: The specificity of the method was studied by analyzing standards and formulation by injecting three times. The peaks of Boswellic acid in formulation were confirmed by comparing retention time (R_t) values with that of reference standard (World Health Organization, 1999).

Accuracy: To check the accuracy of the method, recovery measurements were performed by the addition of the standard drug solution at three different levels (50, 100, and 150%) to the pre analyzed sample solution (20, 40 and 60 µg/ml) in HPLC, Where as HPTLC pre analyzed sample solution (20, 40 and 60 mg/ml) band, so that after the addition of standards, the samples would be in the linear range. Three replicate estimations were carried out for each concentration level in both HPLC and HPTLC. The percentage recovery of standard from the proposed method was calculated.

Precision: To study intraday and inter day precision, three different concentrations of standard solutions were prepared (10, 30 and 50 µg/ml) and injected into HPLC. In case HPTLC three different concentrations of standard (20, 40, and 60 mg/ml) and applied to the TLC plates. All the solutions were analyzed in triplicate on the same day and on different day to record intraday and inter day variations respectively. The percentage recovery of standard from the proposed method was calculated.

Repeatability: The repeatability of HPLC method was assessed by injecting 10, 20 and 30 µl/ml of standard respectively in triplicate (n=3) as per proposed chromatographic conditions.

Table1. Percentage of Assay of Boswellia

S.No	Marketed formulation Name	HPLC		HPTLC	
		Mean±SD (n=3)	RSD%	Mean±SD (n=3)	RSD%
01	Shallaki tablet	76.2432±0.773	1.2861	76.953±0.0044	1.5588
02	Sallaki tablet	78.196±0.0543	1.5563	78.49±0.0087	1.3774
03	Shallaki Hills capsules	75.625±0.007	1.3997	75.117±0.132	1.2932
04	Boswellia Capsules	75.487±0.0994	1.6398	75.998±0.0061	1.1559
05	Boswellia powder	76.26±0.0248	1.4301	75.002±0.092	1.1341

Table 2. Linearity, LOD, LOQ and Range of boswellic acid

Boswellic acid	Linear Range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Slope	Intercept	r^2
HPLC	10-60	30.444	37.5	1100.4	11167	0.9996
HPTLC	10-60	22.72	75.74	211.5	1602.1	0.9928

Table 3. Accuracy and percentage recovery of boswellic acid

S.No	Concentration (µg/ml)	HPLC		HPTLC	
		Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)	Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)
01	20	3621.83±0.98 (0.041)	98.15±0.035 (0.00463)	3621.83±0.98 (0.041)	98.15±0.035 (0.00463)
02	40	6212±1.0020 (0.0016)	98.87±0.5889 (0.00851)	6212±1.0020 (0.0016)	98.87±0.5889 (0.00851)
03	60	8931.44±1.530 (0.010)	98.78±0.019 (0.00996)	8931.44±1.530 (0.010)	98.78±0.019 (0.00996)

Table 4. Intra-day and inter day Precision and percentage recovery of boswellic acid by HPLC

S.No	Concentration (µg/ml)	Intra-day Precision		Inter-day Precision	
		Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)	Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)
01	10	34186.67±1.12 (0.003283)	98.56523±0.31 (0.0324)	34186.67±1.12 (0.003675)	98.49656±0.31 (0.01863)
02	30	85696.5±0.99 (0.001165)	98.7424±0.12 (0.053)	85697.46±0.98 (0.001154)	98.74411±0.12 (0.09354)
03	50	106915.4±1.12 (0.001056)	98.82567±0.12 (0.09523)	106915.4±1.12 (0.001146)	98.7788±0.114 (0.02247)

Table 5. Intra-day and inter day Precision and percentage recovery of boswellic acid by HPTLC

S.No	Concentration (µg/ml)	Intra-day Precision		Inter-day Precision	
		Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)	Mean area ± SD (RSD %)	Percentage recovery ± SD (RSD %)
01	20	3259.22±0.56 (0.017)	98.32±0.022 (0.0011)	3396.83±0.99 (0.044)	98.15±0.0352 (0.0011)
02	40	6110.44±0.99 (0.016)	98.87±0.035 (0.00463)	6122.43±1.02 (0.0036)	98.87±0.5889 (0.00655)
03	60	9268.11±1.005 (0.011)	98.49±0.01586 (0.00952)	9367.12±0.99 (0.085)	98.78±0.015 (0.000544)

The average, standard deviation (SD) and percentage relative standard deviation (% RSD) of peak area was calculated. The repeatability of sample application was in HPTLC assessed by spotting 10 µL of 200,400 and 600 ng/spots of standard respectively on TLC plate (n=3).The plate was developed and scanned as per proposed chromatographic conditions. The average, standard deviation (SD) and percentage relative standard deviation (% RSD) of peak area was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ): To estimate the limits of detection (LOD) and quantification (LOQ), the calibration curve plotted was used for determination of LOD and LOQ. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.LOD and LOQ were calculated using the equations:

$LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma /S$

Where, σ is the standard deviation of the response taken as a measure of the noise and S is the slope of the corresponding calibration plot.

RESULTS

Assay of Boswellia tablets, capsules and powder: The proposed method was successfully applied to the analysis of marketed products (selected powders, tablets and capsule) by HPLC and HPTLC, and the results obtained are given in Table 1.

HPLC Method Optimization: Different compositions of the mobile phase were attempted from earlier reports and the desired resolution of the Boswellic acids with symmetrical and reproducible peaks and a stable base line was achieved by HPLC method performed by with few modifications. In brief the mobile phase used was methanol: Acetontirlie: water (45:2.5:52.5) at UV detection 274nm with a flow rate of 1.0 ml/min. Peak corresponding to 3-Acetyl-11-keto-beta-boswellic acid were symmetrical, sharp, wells resolved and reproducible with retention times at 5.9 min. Linear regression data showed a good linear relationship of 3-Acetyl-11-keto-beta-boswellic acid as shown in the (Figure: 3.3) and over the

concentration range of 10-60 µg/ml of 3-Acetyl-11-keto-beta-boswellic acid with calibration curve of Y= 1100.4x + 1167 and correlation coefficient (r^2) was found to be 0.9996.

HPTLC Method Optimization: Different compositions of the solvent systems were attempted from earlier reports and the distinct separation of the Bosweallic acids was achieved by HPTLC method performed by with few modifications. In brief the solvent system used was Hexane : Ethyl acetate (7 : 3) at UV detection 254nm. Spots corresponding to 3-Acetyl-11-keto-beta-boswellic acid were distinct, well separated. Linear regression data showed a good linear relationship of 3-Acetyl-11-keto-beta-boswellic acid in the concentration range of 10-60mg/ml of 3-Acetyl-11-keto-beta-boswellic acid with calibration curve of Y= 211.1x + 1602 and correlation coefficient (r^2) was found to be 0.9928.

Validation of HPLC and HPTLC Results

Linearity, Range, LOD, and LOQ: The calibration curve of Boswellic acid was plotted using peak area v_s concentration. The method has shown good linearity for Boswellic acid, in the concentration range of 10 - 60 µg/ml (200 - 1000 ng/ml in HPTLC). The LOD, LOQ, slope and intercept were mentioned in the (Table 2).

Accuracy: Accuracy of the method was ascertained by recovery method. The samples of Boswellic acid were prepared in triplicate (n = 3) and analysis was carried out by the proposed method in both HPLC and HPTLC. The percentage recoveries along with SD and RSD values were calculated and mentioned in the (Table 3).

Precision

Intra-day and* inter-day *precision: To determine intra-day and inter-day precision, three different concentrations of Boswellic acid were prepared in triplicate (n = 3) and analyzed at specific time intervals in the same day and different day. The SD and RSD were calculated and mentioned in the (Table 4 and 5).

DISCUSSION

In the present study, an optimized RP-HPLC and HPTLC validated method was established for quantification of Boswellic acid, the major constituents of Boswellia herbal formulations. When see the assay values of herbal formulation of Boswellia (shown in table 1), the test samples is not valid unless the relative standard deviation (RSD) for the replicate injections is not more than 2.0 percent. All five selected powders, tablets and capsule of marketed Boswellia formulation have passed the test because RSD values found to be less than 2.0%. Estimation of Boswellic acid carried out by the HPLC and HPTLC methods. in HPLC analysis mobile phase used as methanol: Acetontirlie: water (45: 2.5: 52.5) at UV detection 274nm with a flow rate of 1.0 ml/min. Peaks corresponding to Boswellic acid were symmetrical, sharp, well resolved and reproducible with retention time as 5.9 min. By this view we can say method is optimized. Similarly in HPTLC also shows the symmetrical, sharp, well resolved and reproducible with retention time of Boswellic acid. In both optimized RP-HPLC and HPTLC methods, the %RSD of regression coefficient was found to be very less (<2%), which is within in the limits of ICH guidelines and the method's linearity was proved to be reproducible. The SD and RSD of Boswellic acid were less than 2% which is the limit as per ICH guidelines, which suggested that the present developed method is accurate. All the three different concentrations of Boswellic acid were shown (table 4 and 5) insignificant RSD and SD which indicates that the both optimized RP-HPLC and HPTLC methods has good repeatability and hence the method proved to be precise.

Conclusion

Determination of Boswellic acid in Boswellia marked herbal formulation carried out and the results suggested that all the five selected herbal formulation posses Boswellic acid within the limits. The proposed HPLC and HPTLC methods can be used for determination of Boswellic acid in various commercial samples for quality evaluation. The method is very simple, rapid and reproducible for rapid screening.

Conflict of interests: The authors declared no competing interests.

REFERENCES

Pallavi P, Shahbaaz S, Sanjar A. 2014. Formulation and Evaluation of Herbal Gel of *Boswellia Serrata* for the Management of Gout. *International Journal for Pharmaceutical Research Scholars,* 3(3): 242-250.

Pawar RK, Sharma S, Singh KC, Sharma RK. 2011. Physico Chemical Standardisation and development of HPTLC method for the determination of β- boswellic acid from *Boswellia serrata Roxb. International Journal of Applied Pharmaceutical,* 3(1), 8-13.

Preeti G, Aakash D. 2015. Anti-Cancer Potential Of Boswellic Acid: A Mini Review; *Hygeia journal for drugs and medicines,* 7(2):18-27.

Reich E, Schibli A. 2007. HigH-PerformancE ThiN-LayeR ChromatographY for the Analysis of Medicinal Plants. U.S.A, 2007; 1, 18-30.

Reshma A, Sadhana JR, Rudresh RR. 2013. Development and Validation of RP-HPLC method for determination of Boswellic acid and Myristicin in Commercial Herbal Formulation. *International Journal of Pharmacy and Pharmaceutical Sciences,* 5(1) 379-383.

Shailesh AS, Ishwarsinh SR, Bhanubhai NS, Saurabh SP, Vijay KP. 2007. Estimation of Boswellic acid from market formulations of *Boswellia serrata* extract and 11-keto-β-boswellic acid in human plasma by high-performance thin-layer chromatography. *Journal of chromatography B,* 848 232-238.

Shailesh AS, Ishwarsinh SR, Bhanubhai NS, Saurabh SP, Vijay KP. 2008. A simple high performance liquid chromatography method for estimation of Boswellic acid from marketed formulations containing *Boswellia serrata* extract. *Journal of chromatography sciences,* 4(8), 1-4.

Shirwaikar AA, Annie S, Aswatha R, Upadhyay DK. 2005. Formulation and Evalution of *Boswellia serrata* tablet ; *Indian Journal of Pharmaceutical Sciences,* 67(4): 427-431.

Shirwaikar AA, Annie S, Aswatha R, Upadhyay DK. 2005. Formulation and Evalution of *Boswellia serrata* tablet ; *Indian Journal of Pharmaceutical Sciences,* 67(4): 427-431.

Sultana A, Khaleeq UR, Padmaja AR, Shafeeq UR. 2013. *Boswellia serrata Roxb.* A Traditional Herb with Versatile Pharmacological Activity. *International Journal of Pharmaceutical Sciences and Research,* 4(6): 2016-2117.

Sunita YR, Ram SG. 2013. Current Strategies In Herbal Drug Delivery For Arthritis. *International Journal of Pharmaceutical sciences and Research,* 4(10), 3782- 3794.

Vivek C, Anu KS. 2016. Formulation And Optimization Of *Boswellia serrata* Extract Tablet ; *Indo American Journal of Pharmaceutical Research,* 6(3), 4899-4910.

Vivek C, Anu KS. 2016. Formulation And Optimization Of *Boswellia serrata* Extract Tablet; *Indo American Journal of Pharmaceutical Research,* 6(3), 4899-4910.

World Health Organization, 1999. WHO Monographs on Selected Medicinal Plants. *Geneva*, Vol- 4, 48-57.

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