Extraction, Identification, Analytical Method Development and Validation of Boswellic acid using UV Spectrophotometric Method

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Abstract

Background: Boswellic acid extracted from *Boswellia serrata* is used as multifunctional drug and one of its uses is in wound healing. Identification of boswellic acid was done by thin layer chromatography and various chemical tests. A simple UV spectroscopic analytical method is developed and validate for the determination of boswellic acid which is not reported yet. The current official method for boswellic acid assay is titrimetry.

Result: Boswellic acid has only one double bond in its structure and thus maximum absorption wavelength was found with difficulty at 247 nm, but still in the developed method correlation coefficient ($R^{2)}$ was 0.9992 and Beer's law was followed in the concentrations between 50-250 µg/ml. followed The concentration range in which Beers's law was is on higher side owing to the fact that boswellic acid has only one double bond, still it is much sensitive than titrimetry. It was discovered that the limits of detection (LOD) and quantification (LOQ) were 17.61515 µg/ml and 53.37923 µg/ml, respectively. The drug's % RSD was found to be less than 2.

Conclusion: The analytical approach was found to be specific, precise, linear, accurate, robust, and can be routinely used for the identification and estimation of boswellic acid alone and in formulations in place of official titrimetric method.

Keywords: *Boswellia serrata*, Boswellic acid, wound healing, anti-inflammatory,TLC, UV method development, validation.

Background

Boswellia is a genus, family Burseraceae and more than 600 species of this genus are spread all over the topical region [1]. Some widely known species of boswellia are mentioned in table1. Basically boswellic acids are extracted from the stem and branches of various Boswellia species. Boswellia tree not just contain boswellic acid but various other constituents are also present like oleo gum which contains resins (30–60%), essential oils (5–10%) and water-soluble polysaccharides (~65% arabinose, galactose, xylose). The resinous part contains monoterpenes (a-thujene), diterpenes (incensole, incensole oxide, iso-incensole oxide, and the diterpene alcohol serratol), triterpenes (a- and b-amyrins), tetracyclic triterpenes (tirucall-8, 24-dien-21-oic acids) and pentacyclic triterpenes. All these pentacyclic triterpenes have multiple pharmacological effects [2][3]. Four major pentacyclic triterpenic acids are α-boswellic acid, β-boswellic acid, 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid

(AKBA) [4][5][6]. The quantity and chemical constituents of that resin changes with various factors like geographical area, procedure of extraction and collection, storage conditions,

season in which extraction process taking place, plant part from which that resinous gum is extracted. Good quality of extrudate is obtained from tree after 3 years of plantation.

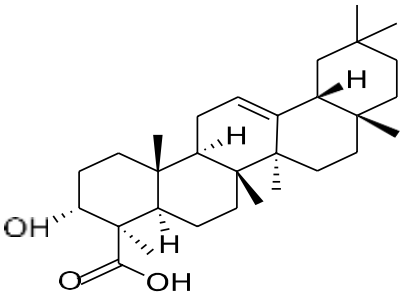

Fig 1 - Structure of boswellic acid

Sr.No.	Species name	Geographical area
1.	*B. Serrata roxeb*	India
2.	*B. Sacra flueck*	Nubia, South Arabia
3.	*B. Bhau-dajiana birdw*	North Somalia
4.	*B.Papyrifera hocst*	Ethiopia
5.	*B. Cateri birdw*	Somalia
6.	*B. Frereana birdw*	Somalia
7.	*B. Neglecta s. moore*	Somalia
8.	*B.Odorata hutch*	Tropical Africa
9.	*B.Dalzielli hutch*	Tropical Africa

Table 1- Widely known species of Boswellia [7][8]

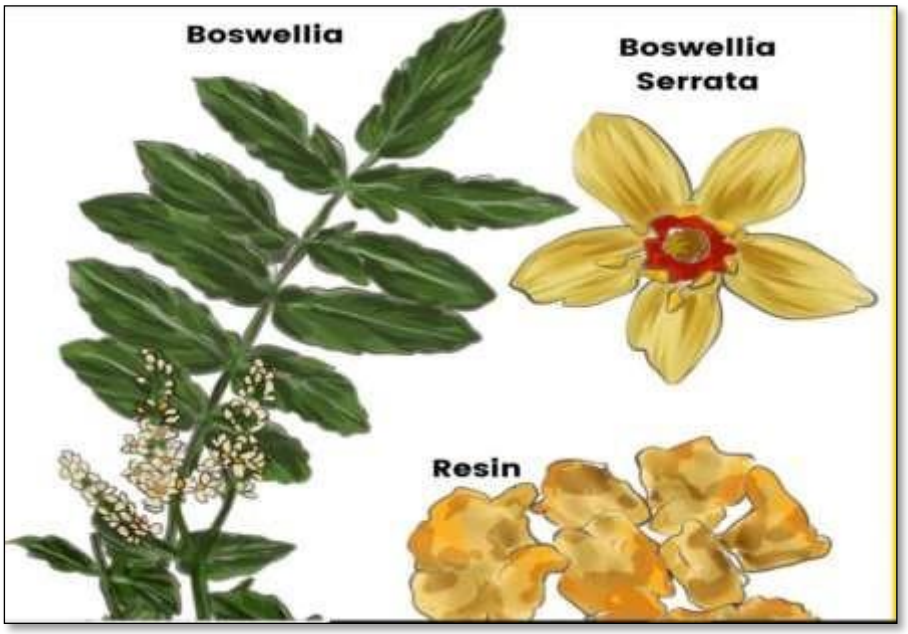

Fig 2 *B. Serrata roxeb*- India

There are various pharmacological actions of boswellic acid such as the traditional application like treatment of GI diseases (Vomiting, diarrhea, constipation and flatulence) and respiratory complications (cough, cold, asthma, bronchitis, dyspnea and hoarseness). Others are modern medicinal applications such as anti-inflammatory property, antidiabetic property (proven in preclinical studies), anticancer activity (cytostatic, anti-apoptotic and antitumor property results in improvements in leukemia, melanoma, hepatoma, prostate and breast cancers, AKBA having effect in inhibition of prostate tumor), antiulcer effects, antimicrobial action on many bacteria including ATCC strain, antithrombolytic effect (hemostatic and cardiovascular effect-having reference in ayurvedic medicine), antifibrotic effect and immune response. All these pharmacological effects are attributed to boswellic acid from exudates of boswellia plant so the extraction and purification of boswellic acid from boswellia plant exudate is must [9].
Standard method of evaluation of boswellic acid is not present as well as need for purification of boswellic acid from resinous matter because commercially boswellic acid is available as 60% in *Boswellia serrata* resins, and literature survey during this research stated that there is no UV spectroscopic method development yet done for this molecule. So project was undertaken .This paper presents extraction, identification, method development and validation of boswellic acid from *Boswellia serrata* resins.

Material and methods

Material

Boswellia serrata (contains 60% boswellic acid) was obtained as a gift sample from Sunpure Extracts Pvt Ltd., Delhi, India. Boswellic acid capsules were procured from local pharmacy. All the reagents were of analytical grade. Double distilled water was used in the entire experiment. Jasco V-730 spectrophotometer with 1 cm matched quartz cells were used for the estimation.

Description of raw material

Boswellia serrata resins were butterscotch yellow in colour and amorphous in nature, resins showed insoluble nature with most solvents.

Extraction of boswellic acid

The resinous matter of *Boswellia serrata* available as a gift sample from Sunpure Extract Pvt Ltd contains boswellic acid(60%). 5 g of *Boswellia serrata* resin was put into the 25 ml of methanol under magnetic stirring for 2 hours, some amount of sample was dissolved in methanol but the other resinous matter remained as a solid residue. This mixture was filtered through whatman filter paper; Filtrate was kept to undergo natural drying. After drying solid material was collected and was subjected to various confirmatory tests for the verification of desired component.

Fig 3 – Extracted boswellic acid from *Boswellia serrata* resins

Identification

Identification of boswellic acid was done by thin layer chromatography. Thin layer chromatography ready-made plates were used as stationary phase, Mobile phase was toluene : ethyl acetate in ratio (9.3:0.7). Mobile phase was poured into the TLC chamber and to maintain humidity and complete saturation filter paper moistened with mobile phase was placed inside the chamber. TLC plates were spotted with the sample concentration 10 ppm (Boswellic acid dissolved in methanol) and placed in the saturated chamber with closed lid. The spots were well above the level of mobile phase. Once the spots were developed, the plates were taken out and dried and further exposed to iodine in a chamber. The plates now were observed under UV light chamber. After detection of spot R_f (Retention factor) value was calculated

$$R_f = \frac{\text{Distance travelled by compound (spot)}}{\text{Distance travelled by solvent}}$$

4 spot were observed at a distance 1.5cm, 2cm, 3.3cm, 4cm and distance travelled by solvent was 5.5cm. R_f values were found to be 0.27, 0.36, 0.6, 0.72 [10][11].

Fig 4 – Identification of boswellic acid -TLC plate under UV light chamber

Confirmatory tests

Boswellic acid has steroidal structure so following tests were tried for confirmation.

1. Liebermann-Burchard tests – 10 mg Sample extracted from *Boswellia serrata* was added in a 1 ml chloroform and shaken to dissolve. Acetic anhydride and sulphuric acid was added into above solution.
2. Salkowski test – 10 mg Sample extracted from *Boswellia serrata* was added in 1 ml chloroform and shaken to dissolve. Concentrated sulphuric acid was added into above solution.

Table 2- Confirmatory chemical tests for boswellic acid

Sr.No.	Tests	Results	Conclusion
1.	Liebermann-Burchard test	Green colour formed	Boswellic acid confirmed
2.	Salkowski test	Red colour formed	Boswellic acid confirmed

From these confirmatory tests it can be concluded that extract of *Boswellia serrata* contains boswellic acid and boswellic acid is preferentially dissolved in methanol compared with other ingredients present in Boswellia *serrata* resin [12].

Preparation of standard stock solution of boswellic acid-

An accurately weighed 10 mg of boswellic acid was dissolved in 10 ml of methanol in a 10 ml volumetric flask to obtain a stock solution of 1000 µg/ml. The solution of boswellic acid was filtered through Whatman filter paper no. 41 [13].

Determination of λmax

An appropriate aliquot portions of 0.5 to 2.5 ml of stock solution were transferred to a series of 10 ml volumetric flasks and volume in each flask were adjusted to 10 ml with methanol to obtain a concentration of range of 50-250 µg/ml. One of the solutions was scanned in UV range of 200-400 nm using methanol as a blank and wavelength of maximum absorption was found to be 247 nm. The absorbance of solutions was measured at 247 nm against blank and calibration curve of boswellic acid was constructed [13].

The wavelength of maximum was confirmed for marketed sample of boswellic acid capsule as external standard. It matched the wavelength of maximum of 247 nm

Assay of marketed capsule formulation of boswellic acid.

Twenty capsules containing 500 mg boswellic acid were broken into 2 parts cap and body and powder of boswellic acid was collected. Amount equivalent to 10 mg was transferred to 10 ml volumetric flask and was dissolved in 10 ml of methanol to obtain a concentration of 1000 µg/ml. The boswellic acid powder solution was filtered through Whatman filter paper No. 41 to remove other excipients form capsule as boswellic acid is completely soluble in methanol but excipients were not. Filtrate was diluted to make concentration in between linearity range. The absorbance of sample solution was measured and amount of boswellic acid was determined by referring to the calibration curve [14].

Since boswellic acid present in marketed capsule formulation showed 247nm as λ_{max} and showed the linear response to give a standard calibration curve in methanol, it -confirms that UV spectrophotometric method for boswellic acid as API can be continued and validated.

Validation methods for Boswellic acid (Purified extract from Resin) [13][14]

1. **Linearity**

   Linearity range for boswellic acid at λmax (247nm) wavelength was found to be 50-250 µg/ml. The linearity equation was y = 0.0032x+0.0098 with a correlation coefficient of 0.9992. For examination of five replicate samples, a relative standard deviation of 0.2470 was found, showing that the developed approach is accurate

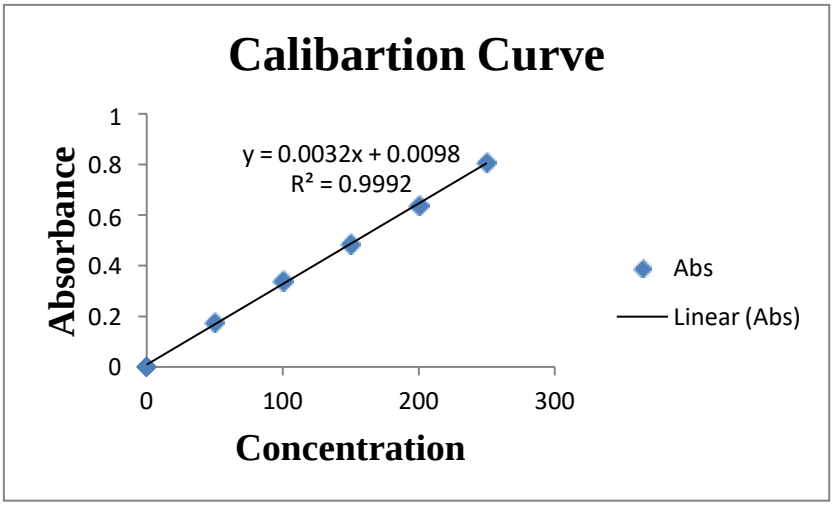

Fig 5 – calibration curve of boswellic acid

2. **Precision**

   Precision of developed method is necessary to check to what degree to which an instrument or process will repeat the same value. It is calculated in terms of % relative standard deviation (% RSD). The % RSD value should be less than 2. These findings suggest that the assay is reproducible. Precision is carried out by intra-day precision and inter-day precision, intra-day was on the same day three times but on different time and inter-day was calculated by every day for three days of the week

Concentration (µg/ml)	**Intraday precision (n = 3)**		**Interday precision (n = 3)**	
	Conc. Found	**% RSD**	**Conc. Found**	**% RSD**
150	148.06	0.4177	149.18	0.3599
200	196.34	0.1600	199.18	0.5117
250	246.68	0.4398	245.96	0.0815

Table 3 – Precision result for boswellic acid estimation

3. Accuracy

The accuracy was tested at 150 µg/ml by standard addition method where, standard solution concentrations of 80%, 100%, and 120 percent. Area under curve (AUC) was measured in wavelength range 247 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 150 µg/ml standard solution. Area under curve (AUC) was measured in wavelength range 247 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Accuracy level	**Sample conc. (µg/ml)**	**Std. conc.**	**Total Amount. Added (µg/ml)**	**% Recovery**	**Mean % Recovery**	**% RSD**
80	150	120	270	98.55		
100	150	150	300	99.61	99.93667	1.5766
120	150	180	330	101.65		

Table 4 – Accuracy result for boswellic acid

4. LOD and LOQ

The sensitivity of a method is determined by its limit of detection (LOD) and limit of quantification (LOQ). LOD is used to calculate the sample's lowest detectable concentration, while LOQ is used to calculate the sample's minimum measurable concentration.

Limit of detection = 3.3 δ/s Limit of quantification = 10 δ/s

The limit of detection (LOD) and limit of quantification (LOQ) were found to be 17.61515 µg/ml and 53.37923 µg/ml

5. Summary of Validation Parameters

Parameter	**Values**
λ_{max} (nm)	247
Beer's range (µg/ml)	50-250
Correlation coefficient (r2)	0.9993
Regression equation	y = 0.0032x + 0.0098
Intercept (a)	0.0098
Slope (b)	0.0032
Limit of detection (LOD µg/ml)	17.61515
Limit of quantification (LOQ µg/ml)	53.37923

Table 5 - Summary of Validation Parameters

Result and discussion

- Boswellic acid from *Boswellia serrata* resin was extracted and identified
- After TLC identification, 4 spots were observed at a distance 1.5cm, 2cm, 3.3cm, 4cm and distance travelled by solvent was 5.5cm, and R_f values were found to be 0.27, 0.36, 0.6, 0.72 compared with R_f values of 11-keto-boswellic acid (0.27) and Acetyl-11-keto-boswellic acid (0.36) , with two additional components.
- Sample of boswellic acid was confirmed with chemical tests of steroids.
- Maximum absorbance of extracted boswellic acid was found at wavelength 247 nm (λ_{max})
- The drug concentrations were discovered to be linear in the 50–250 µg/ml range
- The linearity of the established method is indicated by the correlation coefficient value of 0.9992
- The intra-day and inter-day precision results for concentrations 150, 200, and 250 in terms of percent relative standard deviation values were determined to be 0.4177, 0.1600, 0.4398 and 0.3599, 0.5117, and 0.0815. The precision % RSD is less than (<2) which conforms with the standard.
- In case of accuracy study, % recovery study was determined which was near 100% and % RSD value was less than 2 (1.5766)
- The values for the limit of quantitation and the limit of detection were discovered to be 17.61515 µg/ml and 53.37923 µg/ml

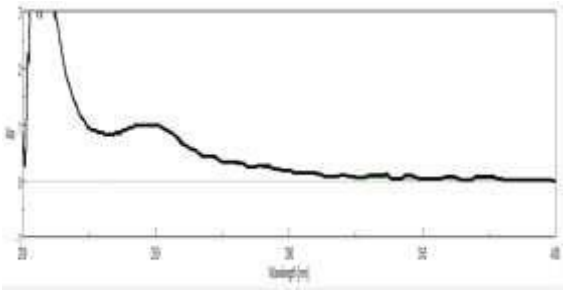

Fig 6 - UV Spectrum of boswellic acid

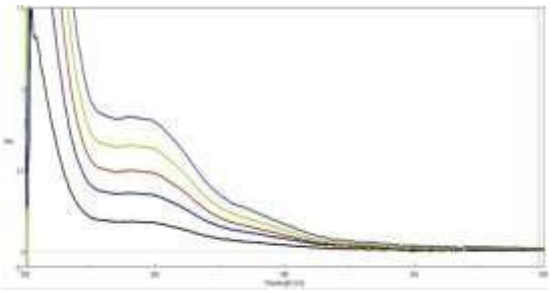

Fig 7 – Overlay of boswellic acid for Concentration 50-250 µg/ml with λ_{max} of 247 nm

Conclusion

The UV Spectroscopic method for analysis of Boswellic acid was in accordance with ICH Q2 (R1) guidelines and it satisfies acceptance standards. The analytical approach was found to be specific, precise, linear, accurate, robust, and can be used for assay of boswellic acid as bulk drug and pharmaceutical dosage formulations containing boswellic acid. The current analytical technique is suitable for the intended applications.

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