

**SAMI LABS LIMITED**

19/1 & 19/2, I Main, II Phase, Peenya Industrial Area, Bangalore – 560 058

STANDARD TEST PROCEDURES

No.: SLL/STP-B-002		Page 1 of 4
Title: HPLC METHOD FOR ESTIMATION OF β -BOSWELLIC ACIDS AND ASSAY BY TITRATION		
Issue: 5	Supersedes: Issue 4 dated April 16, 2015	Effective: March 05, 2018

Objective : To analyze the content of β -Boswellic acids by HPLC

Scope : The procedure is applicable to all the Analytical divisions and quality control divisions of Sami Labs Limited.

Responsibility : Analytical Chemist / Analyst

Accountability : Laboratory In-charge

Reagents required:

- Acetonitrile (HPLC Grade or equivalent)
- Methanol (HPLC Grade or equivalent)
- Water (Double distilled)
- Glacial Acetic acid (AR Grade or equivalent)

Mobile phase:

- Mix Acetonitrile and Water in the ratio of 90:10 with 0.05mL of Glacial Acetic acid.
- Filter and sonicate.

Diluent: Methanol

	Prepared by	Checked by	Approved by
Signature			
Name	Ramesha SE	Mubarak Pasha	Dr. Satish N. Sawant
Designation	Documentation Executive	Sr. Manager	Vice President
Department	Analytical R & D	Analytical R & D	Analytical R & D
Date	05/03/2018	05/03/2018	05/03/2018

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Chromatographic conditions:

Column : C18 packing(Merck C18 or YMC C18).
4.6mm x 250 mm or equivalent
Mobile phase : As mentioned above
Flow rate : 1.0 mL/min
Detector : UV/VIS (210nm & 254nm)
Injection volume : 20 μ L

Flow rate:

<u>Time (Minutes)</u>	<u>Flow (mL)</u>
01	1.0
05	1.5
10	2.0
30	2.0
32	1.5
35	1.0

Standard preparation:

- Transfer about 200 mg of the working standard of β -Boswellic acids, accurately weighed, to a 50mL volumetric flask.
- Add about 20 mL of Methanol to dissolve.
- Sonicate for about 5 minutes.
- Dilute to volume with Methanol and mix.

Sample preparation:

- Weigh sample accurately equivalent to 200mg of standard to a 50 mL volumetric flask.
- Add about 20 mL of Methanol.

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- Sonicate for about 5 min.
- Make upto the volume with Methanol and mix.

Procedure:

- Start HPLC as per the chromatographic conditions and equilibrate the column with mobile phase.
- Inject 20 μ L of Methanol as blank.
- Inject 20 μ L of standard preparation in five replicates.
- Inject 20 μ L of sample preparation in duplicate.
- Inject 20 μ L of standard preparation as bracketing standard.
- Ignore any peaks due to blank in sample and standard chromatogram.
- Record the responses of major peak due to analyte.

System suitability:

- The relative standard deviation (% RSD) for peak area and peak retention time for five injections of standard preparation should not be more than 2 % & 1 % respectively
- The theoretical plate count of the analyte peak in the standard preparation should not be less than 2000 and the tailing factor should be less than 2.0

Order of Elution at 210nm β -Boswellic acidsAcetyl- β -Boswellic acids**Order of Elution at 254nm**Keto- β -Boswellic acidsAcetyl-keto- β -Boswellic acids

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Calculation:

Area of β -BA sample x Standard concentration in mg / ml x Potency of β -BA in standard

Area of β -BA standard x Sample concentration in mg / ml

Same way calculate for acetyl β -Boswellic acid, keto- β -Boswellic acid and acetyl-keto- β -Boswellic acid.

BA = Boswellic acid

Note: This method is applicable for all the grades of Boswellic acids

Assay by Titration**UNCONTROLLED COPY**

- Weigh accurately about 0.75 g of the sample
- Dissolve it in 100 mL of neutralized Alcohol.
- Titrate against 0.1 N NaOH using Phenolphthalein solution as the indicator.

Calculation:

Titre value x 0.0456 x Normality of 0.1 N NaOH x 100

Wt. of sample x 0.1

Method reference: USP 34

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