STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS
Background & Fitness for Purpose
Aloe vera

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Background on Analyte

- Polysaccharides are one of the major constituents in Aloe vera.
- The major polysaccharide in aloe is glucomannan which is consisted of mannose (major) and glucose (minor) with 1,4-β-linked backbone. The mannose moieties are highly acetylated and are referred to Acemannan in literature.
Background

- Aloe leaf consists of an outer green rind (skin) and an inner clear pulp;

Rind

Inner pulp

- Both rind and inner pulp contain polysaccharides;
- Other major components in aloe leaf are organic acids, minerals, and monosaccharides;
- Rind contains four major organic acids, malate, isocitrate, isocitrate lactone, and citrate, while pulp contains major malate and some citrate, but very minimal isocitrate and isocitrate lactone;
Background

• Based on the parts to be used, aloe leaf products can be divided into:
  – Entire leaf juice;
  – Inner leaf juice;
• Based on manufacturing process, aloe products can be divided into:
  – Enzymatic treatment;
  – Non-enzymatic treatment;

• Accepted criteria by aloe industry, the average solid contents in the inner gel are about 0.5% and entire leaf 1%.
• The concentration of aloe product is expressed:
  – 5X: 5 Time concentrated comparing with fresh aloe leaf;
  – 200X: Inner leaf extract (200 parts of inner gel to make 1 part of powder);
  – 100X: entire leaf extract (100 parts of entire leaf to make 1 part of powder) or 200X is diluted with 50% excipients;
Significance

• Acemannan is reported to be response to biological activities of *Aloe vera* including:
  – Immunostimulatory;
  – Anti-inflammatory;
  – Hypoglycemic and hypolipidemic activities;
  – Antibacterial, antiviral, and antitumor effects;

General Analytical Needs

• Method should:
  – Qualitatively identify aloe polysaccharides;
  – Quantitatively determine aloe polysaccharide contents;
  – Determine molecular weight of aloe polysaccharides;
  – Differentiate aloe product type, entire leaf vs. inner leaf;
Challenges

- A single method to meet all the requirements;
- Exclusive to aloe polysaccharides;
- Accurately quantitate aloe polysaccharides;
- Discrepancies of aloe polysaccharide structures reported in literature;

Existing Methods - General

- HPTLC
- Colorimetric Assay by Red Dye
- Colorimetric Assay of Acetyl Groups of Polysaccharides
- $^1$H NMR Spectroscopic Method
- Size Exclusion Chromatography
Existing Methods

• HPTLC

  ![HPTLC chromatogram of A. vera gel](from the above paper)

  **HPTLC conditions:**
  Plate: Si-gel Si60F254
  Reagents: anisaldehyde sulphuric acid reagent; heating the plate at 105–110°C for 5 min.

• Colorimetric Assay by Red Dye – Congo Red

  ![Congo red dye and Aloe polysaccharides](

• Complex of Congo red with polysaccharides and assayed at 540nm
Existing Methods

• Colorimetric Assay by Red Dye – Alizarin Red

  ![Alizarin Red](image)

  + Aloe polysaccharides

• Complex of alizarin red with polysaccharides and assayed at 325nm and 516nm

Existing Methods

• Colorimetric Assay of Acetyl Groups of Polysaccharides
  – Aloe Products for Food Raw Material, Chinese National Standard, QB/T 2489 2007

  ![Acetohydroxamic Acid](image)

• The acetyl groups on polysaccharides are reacted with hydroxylamine to form acetohydroxamic acid. The resulted acetohydroxamic acid is reacted with ferric trichloride to form a ferric-acetohydroxamic acid complex and measured 540nm.
Existing Methods

- \(^1\)H NMR Spectroscopic Method
Existing Methods

- Size Exclusion Chromatography

![Graph](image)

Fig. is from the above paper

Regulatory Guidance

- No information regarding the determination of aloe acetyl polysaccharide contents in the following pharmacopoeias:
  - United States Pharmacopeia;
  - European Pharmacopoeia
  - Chinese Pharmacopoeia;
  - Japanese Pharmacopoeia;
Fitness for Purpose (proposal)

- The methods are able to qualitatively identify aloe vera;
- Are able to accurately quantitate not only the contents of aloe polysaccharides, but also the molecular weight;
- Are able to accurately quantitate the aloe polysaccharides with different molecular weight;

QUESTIONS??