QUILLAIA EXTRACT (TYPE 2)

Revised specifications prepared at the 65th JECFA and published in FNP 52 Add 13 (2005), superseding specification prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). A group ADI of 0-1 mg quillaia saponins /kg bw for Quillaia ExtractsTypes 1 & 2 was established at 65th JECFA (2005)

SYNONYMS Quillaja extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract; INS No. 999(ii)

DEFINITION Quillaia extract (Type 2) is obtained either by chromatographic separation or ultrafiltration of the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are minor components. Some sugars and calcium oxalate will also be present.

Quillaia extract (Type 2) is available commercially as a liquid product or as a spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.

C.A.S. number 68990-67-0

Formula weight Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues

Assay Saponin content:
not less than 65 % and not more than 90 % on the dried basis

DESCRIPTION Light red-brownish liquid or powder

FUNCTIONAL USES Emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, insoluble in ethanol, acetone, methanol, and butanol

Foam Dissolve 0.5 g of the powder form in 9.5 ml of water or 1 ml of the liquid form in 9 ml of water. Add 1 ml of this solution to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam volume (ml) after 30 min. Typical volumes are about 260 ml.

Chromatography Determine as in METHOD OF ASSAY. The retention time of major sample peak corresponds to the major saponin peak (QS-18) of the standard.

Colour and turbidity Powder form only: Dissolve 0.5 g in 9.5 ml of water. The solution shall not turbid Determine the absorbance of the solution against water at 520 nm. The absorbance shall be less than 0.7.

PURITY

Water (Vol. 4) Powder form: not more than 6% (Karl Fischer Method)

Loss on drying (Vol. 4)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (Vol. 4)	3.7 -5.5 (4 % solution)
Ash (Vol. 4)	Not more than 5% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from Loss on drying)
Tannins	Not more than 8% on a dried basis See description under TESTS
Lead (Vol. 4)	Not more 2 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in FNP 5, "Instrumental Methods".

TESTS

PURITY TESTS

Tannins

Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (W_i). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at 800 × g. Recover the supernatant and dry this solution at 105° (5 h).Weigh the recovered solid (W_f, in g). The percentage of tannins in the sample is:

$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle:
The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 2).

Sample preparation:
Powders: Weigh 0.5 g of sample and dissolve in 9.5 ml of water. Filter through a 0.2 µm filter.
Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 ml of water. Filter through a 0.2 µm filter.
In each case, the sample volume is ca. 10 ml.

Standard preparation:
Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 µm filter.

High performance liquid chromatography (HPLC):
HPLC conditions:
Column: Vydac 214TP54 (4.6 x 250 mm length, 5 µm particle size) or equivalent
Column temperature: Room temperature
Pump: Gradient
Solvent A: 0.15% trifluoroacetic acid in HPLC-grade water.
Solvent B: 0.15% trifluoroacetic acid in HPLC-grade acetonitrile.

Gradient:

Time(min)	% solvent A	% solvent B
0	70	30
40	55	45
45	70	30

Flow rate: 1 ml/min
Detection wavelength: 220 nm
Injection volume: 20 µl

Calculation:
The concentration of saponins, C_{sap}, in mg/ml, in the solution prepared as directed under sample preparation is:

$$C_{sap} = (A_{sample}/A_{standard})C_{Standard}$$

where $C_{Standard}$ (mg/ml) is the saponins concentration of the standard injected (e.g., $C_{Standard}$ = 13.5 mg/ml if the saponin content of 1.5 g of standard sample is 90 %) and A_{sample} and $A_{standard}$ are the sums of the peak areas attributed to the four principle saponins in the sample preparation and in the standard preparation, respectively, as noted in the figure. (Tannins and polyphenols will elute before the saponins. The peaks corresponding to the saponins will appear after the major peak corresponding to the polyphenols)

The percentage of saponins in the test sample is:

$$\% \text{ Saponins} = 100 \times C_{sap}/(0.1W_{sample})$$

where W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

Appendix

Chromatogram of Standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).