TANNIC ACID

Prepared at the 39th JECFA (1992), published in FNP Add 1 (1992) superseding specifications prepared at the 35th JECFA (1989), published in FNP 49 (1990) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not specified' was established at the 35th JECFA (1989)

SYNONYMS   Tannins (food grade), gallotannic acid, INS No. 181

DEFINITION   Consists of gallotannins obtained by solvent extraction from certain natural sources; the substance is not an acid in the chemical sense. The common name "Tannic acid" has been adopted to distinguish the commercial substance from other tannins, such as condensed tannins. These specifications relate only to hydrolysable gallotannins, i.e., those which yield gallic acid on hydrolysis. These specifications do not apply to many other kinds of tannins which occur in nature, including condensed (non-hydrolysable) tannins and hydrolysable ellagitannins. Hydrolysable gallotannins may be obtained from nutgalls, the excrescences which form on young twigs of various *Quercus* species, e.g., *Q. infectoria*; these include Chinese and Aleppo tannins. They may also be obtained from various Sumac species, e.g. *Rhus corieria*, *R. galabra*, *R. thypia*; these include Sicilian and American sumacs. All of these consist essentially of polydigalloyl esters of glucose. A further source of hydrolysable gallotannins is the seed pods of Tara (*Caesalpinia spinosa*); these tannins consist essentially of the polydigalloyl esters of quinic acid.

Assay   Not less than 96% on a dried basis

DESCRIPTION   Amorphous powder, glistening scales or spongy mass, varying in colour from yellowish white to light brown; odourless or with a faint, characteristic odour

FUNCTIONAL USES Clarifying agent, flavouring agent, flavour adjunct

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)   Soluble in water, acetone and ethyl alcohol; insoluble in benzene, chloroform and ether; 1 g dissolves in about 1 ml of warm glycerin

Colour reaction   To a 1 in 10 solution add a small quantity of ferric chloride TS. A bluish black colour or precipitate forms

Precipitate formation   A solution of the sample when added to a solution of either albumin or gelatin produces a precipitate

Test for gallic acid   Passes test after hydrolysis
See description under TESTS

PURITY

Loss on drying (Vol. 4)   Not more than 7% (105°, 2 h)

Sulfated ash (Vol. 4)	Not more than 1% Test 1.0 g of the sample (Method I)
Gums or dextrin	Dissolve 1 g in 5 ml of water, filter, and to the filtrate add 10 ml of alcohol; no turbidity is produced within 15 min
Resinous substances	Dissolve 1 g in 5 ml of water, filter and dilute the filtrate to 15 ml; no turbidity is produced
Condensed tannins	Not more than 0.5% See description under TESTS
Residual solvent (Vol. 4)	Not more than 25 mg/kg acetone or ethyl acetate, singly or in combination See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

IDENTIFICATION TESTS

Test for gallic acid	Proceed as directed under *Chromatography (thin-layer chromatography)* (see Volume 4)

Sample preparation:
Add 20 mg of tannase to 100 ml of 1 in 1000 solution of the sample. Incubate at 37° for 2 h.

Standard preparation:
1 in 1000 solution of gallic acid.

Developing solvent:
A mixture of 5 volumes of chloroform, 4 volumes of ethyl formate and 1 volume of formic acid.

Spraying reagent:
A mixture of 1 volume of 1 in 100, 10% ethanol solution of ferric chloride ($FeCl_3$), and 1 volume of 1 in 100, 50% ethanol solution of potassium ferricyanide ($K_3FeCN)_6$).

Absorbent: Silica gel
Apply 10 µl of the sample preparation and 10 µl of the standard preparation. Stop the development when the solvent front has advanced about 15 cm, dry in air, and spray the reagent. The Rf value of the sample and that of the reference standard are identical.

PURITY TESTS

<u>Condensed tannins</u>

The following HPLC method can be used for the determination of condensed tannins in the sample.

<u>Sample preparation:</u>
Dissolve 200 mg of the sample in 100 ml of a mixture of 100 volumes of 10% methanol and 0.1 volume of phosphoric acid.

<u>Standard preparation:</u>
Dissolve 10 mg of rutin in 100 ml of methanol.

<u>Conditions:</u>
Column: Merck Hibar, lichrosolv RP 18 5 μ (Art. 50333), or equivalent.
Column temperature: Room temperature
Pump: gradient
Eluent: A: 0.2% H_3PO_4 in water; B: 0.2% H_3PO_4 in methanol

<u>Gradient:</u>

Time (min)	% A	%B
0	90	10
65	25	75
66	0	100
75	0	100
76	90	10
95	90	10

Elution rate: 1 ml/min.
Detection wavelength: 350 nm
Injection volume: 10 μl

Calculate the content of the condensed tannins as follows:

$$\% \ condensed \ tannins \ (as \ rutin) \ = \ \frac{A \ x \ W_o}{A_o \ x \ W} \ x \ 100$$

Where
A = peak area of the sample (condensed tannins)
A_o = peak area of the reference standard (rutin)
W = weight in mg of the sample taken (200)
W_o = weight in mg of the reference standard taken (10)

<u>Residual solvent</u>

<u>Reagents</u>
- Acetone
- Ethyl acetate

<u>Standard solutions</u>
Place 1 g of acetone and ethyl acetate in a volumetric flask and add water to total volume of 100 ml, and prepare 0.02 - 0.4 g/100 ml solutions by dilution of this solution.
<u>Procedure</u>
Place 1 g (1.0±0.1 g) of powdered sample in a sample vial. Add 5 μl of water to the sample vial and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the below-mentioned conditions.

<u>Calibration</u>
Take 1 g of tannic acid of solvent free or known residual solvent contents in a sample vial, add 5 µl of the standard solution and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the following conditions and obtain calibration curves for each solvent.

Determine by *Gas chromatography*, using a head space sampler under the following conditions:
Column:
- length: 30 m
- diameter: 0.53 mm (i.d.)
- material: silica capillary
- Stationary phase: 100% methyl polysiloxane
- Column conditioning: Heat to 60° for 2-3 h with approximately 10 ml/min of nitrogen
Carrier gas: Nitrogen
Flow rate: 5 ml/min
Detector: Flame ionization
Temperatures
- injection port: 110°
- column: 40°
- detector: 110°

<u>Head space sampler</u>
Sample volume: 1.0±0.1 g + 5 µl
- Sample heating temp.: 80°
- Sample heating time: 40 min
- Syringe temperature: 85°
- Sample gas injection: 0.4 ml

<u>Calculation</u>

$$C_i = A_i \; x \; Cf_i \; x \; 1000$$

where
C_i = concentration of solvent-i (mg/kg)
A_i = peak area of solvent-i (Tv. sec.)
Cf_i = conversion coefficient for solvent-i (slope of the calibration curve) (µg/Tv.sec)

METHOD OF ASSAY

<u>Sample test:</u>
Accurately weigh about 2.0 g of the sample, transfer to a 500-ml volumetric flask, add water to dissolve and make up to the volume with water. Transfer 100 ml of this solution into a 300 ml Erlenmeyer flask and add 7.2 g of Hide Powder (a suitable grade is available from L.H. Lincoln & Son, Inc., Tanning Materials, Coudersport, Pennsylvania, 16915 USA). Shake the flask for 20 min. Let stand for 10 min. and filter through a G_4-filter. The filtrate shall be clear. Pipette 50 ml of the filtrate into a tared crystallizing dish. Evaporate to dryness on a steam bath and heat in an oven at 105° for 1 h. After cooling in a desiccator, weigh again and calculate the weight difference called A.

<u>Blank test:</u>

On each lot of Hide Powder (a suitable grade of Hide Powder may be obtained from L.H. Lincoln & Son, Inc., Tanning Materials, Coudersport, Pennsylvania, 16915 USA) a blank test has to be carried out. 7.2 g of Hide Powder EFT is weighed into a 300 ml Erlenmeyer containing 100 ml water. Proceed as directed for the Sample test, beginning with "Shake the flask for 20 min...". The weight difference is called B.

Calculation

$$\% \; Tannic \; acid \; = \; \frac{(A - B) \; x \; 1000}{W}$$

where
A = weight difference found in the sample test (g)
B = weight difference found in the blank test (g)
W = weight of the sample, on a dried basis (g)