

BENZOE TONKINENSIS (TENTATIVE)

New tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011). No ADI was allocated at the 74th JECFA (2011).

Information required on at least five different batches of commercially available product on the following:

- *Identification of substances other than benzoic acid, coniferyl benzoate, vanillin and benzyl benzoate in the ethanol extract;*
- *Data on microbiological contaminants (E. coli, Salmonella spp);*
- *Data on levels of inorganic contaminants (lead, arsenic, antimony, chromium, mercury and cadmium).*

A method for distinguishing between Benzoe tonkinensis and Sumatra benzoin should also be provided.

SYNONYMS

Siam Benzoin gum; Siam Benzoin Laos; Styrox tonkinensis

DEFINITION

Benzoe tonkinensis is a natural complex balsamic resin obtained from a native tree in Laos: *Styrox Tonkinensis* (Pierre). It is collected directly from the tree, cleaned and sorted into four grades according to size. All grades have similar chemical composition. The resin is composed mainly of benzoic acid (15-45%) and coniferyl benzoate (15-60%). Lesser amounts of vanillin (<5%), benzyl benzoate (<2%), 2-hydroxy-1 phenyl ethanone and 1-(4-hydroxy-3-methoxyphenyl)-2 propanone are also present.

These specifications do not cover Sumatra benzoin (resin obtained from *Styrox benzoin* Dryander and *Styrox paralleloneurum*).

C.A.S. number

9000-72-0

DESCRIPTION

White-yellow to reddish splits of flattened almond-like grains with a strong vanilla smell.

FUNCTIONAL USES

Flavouring agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water; soluble in ethanol

Benzoic acid, vanillin and benzyl benzoate

The sample contains benzoic acid, vanillin and benzyl benzoate identified by their characteristic peaks in the chromatogram. See description under TESTS.

<u>Coniferyl benzoate</u>	The sample contains coniferyl benzoate identified by its characteristic peak in the chromatogram. See description under TESTS.
PURITY	
<u>Loss on drying (Vol. 4)</u>	Not more than 5.0% (105°, 4 h). Test 2 g of sample.
<u>Total ash</u>	Not more than 2.0% Evenly distribute 1.0 g of the powdered sample in a crucible. Dry at 100-105° for 1 h and ignite to constant mass in a muffle furnace at 600 ± 25°.
<u>Alcohol-insoluble matter</u>	Not more than 5%. To 2.0 g of the powdered sample add 25 ml of ethanol 90% v/v. Boil until almost completely dissolved. Filter through a previously tared sintered-glass filter and wash with three 5 ml portions of boiling ethanol 90% v/v. Dry the residue at 100-105° for 2 h and weigh after cooling.
<u>Acid Value (Vol. 4)</u>	Between 160 and 206
<u>Benzoic acid</u>	Between 15 and 45% See description under TESTS
<u>Coniferyl benzoate</u>	Between 15 and 60% See description under TESTS
<u>Lead (Vol. 4)</u>	Not more than 2 mg/kg Triturate the sample in a centrifugal grinder to a particle size < 200 µm. Determine using an AAS or ICP-AES 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 (under "General Methods, Metallic Impurities").
<u>Microbiological contamination</u>	Information required

TESTS

IDENTIFICATION AND PURITY

Benzoic acid, vanillin and benzyl benzoate Benzoic acid, vanillin and benzyl benzoate are identified and benzoic acid is quantified by gas chromatography.

Reagents

Ethanol 96%

Reference standard of benzoic acid (purity >99%)

Reference standard of vanillin (purity > 95%)

Reference standard of benzyl benzoate (purity > 95%)

Gas Chromatographic system

Detector: Flame ionization detector

Column: RESTEK, Rxi-5HT (30 m x 0.25 mm I.D., 0.25 µm-film) or equivalent

Carrier gas: helium

Flow rate: 1 ml/min

Temperatures:

- Injector: 250°

- Oven: 5 min at 80°; to 320° at 10°/min; then hold for 61 min

- Detector: 250°

Injection mode: split 1/10

Run time: 90 min

Benzoic acid elutes at 9.8 min. Peaks at 13.3 and 17.6 corresponds to vanillin and benzyl benzoate, respectively.

Benzoic acid standard stock solution (20 mg/ml): Weigh accurately 0.4 g (± 0.1 mg) of benzoic acid reference standard, transfer to a 20 ml volumetric flask and bring to volume with ethanol.

Benzoic acid standard solutions: Prepare five solutions from the standard stock solutions within in the concentration range of 0.25 to 20 mg/ml.

Vanillin and benzyl benzoate solution: Prepare a 10 mg/ml solution containing vanillin and benzyl benzoate in ethanol.

Sample solution

Accurately weigh about 5.0 g (± 0.1 mg) (w_s) of the previously crushed Benzoe tonkinensis and solubilize in 20 ml of ethanol (96%). Sonicate the mixture and filter. Re-extract the residue with a second portion of 20 ml ethanol (96%), sonicate and filter. Combine the ethanol extracts and evaporate the solvent under vacuum. Weigh accurately the extracted resin (w_{ex}). Dissolve the resin with ethanol to a final concentration of 30 mg/ml.

Procedure

Inject 1 µl of each benzoic acid standard solution and record the peak areas. Inject 1 µl of the 10 mg/ml solution containing vanillin and benzyl benzoate.

Plot a standard curve (concentration of benzoic acid (mg/ml) (X-axis) vs. peak area of benzoic acid (Y-axis)) and determine the slope (m) and the linear coefficient (a).

Inject 1 µl of the sample solution and record the peak area.

For identification of vanillin and benzyl benzoate, compare the retention times of the corresponding peaks of vanillin and benzyl benzoate in the chromatograms obtained with the standard solution and sample solution.

Calculation

Calculate the content of benzoic acid as follows:

$$\text{Benzoic acid (w/w, \%)} = \left(\frac{A_{BZA} - a}{m} \right) \times \frac{1}{30} \times \frac{w_s}{w_{ex}} \times 100$$

where

A_{BzA} is the peak area of benzoic acid in the sample;

a is the linear coefficient of the standard curve;

m is the slope of the standard curve;

w_{ex} is the weight of resin extracted with ethanol (g); and

w_s is the weight of sample Benzoe tonkinensis (g).

Coniferyl benzoate

Coniferyl benzoate is identified and quantified by high performance liquid chromatography.

Reagents

Acetonitrile, HPLC grade

Formic acid

Reference standard of coniferyl benzoate (>95%)

Chromatographic system

HPLC system with a diode array detector (DAD), auto sampler or injector.

Detector wavelength for quantitation: 300 nm

Column: Luna C18 Phenomenex (15 mm x 4.6 mm, 5 μ m) or equivalent.

Mobile phase: solvent A: water added of 0.1% formic acid and solvent B: acetonitrile added of 0.1% formic acid

Gradient elution: A:B 65:35 v/v (0 to 5 min) to A:B 0:100 v/v (5 to 25 min)

Column temperature: 25°

Flow rate: 1 ml/min

Injection volume: 10 μ l

Coniferyl benzoate elutes at 14.9 min.

Standard stock solution (coniferyl benzoate 8 mg/ml): Weigh accurately 0.04 g (\pm 0.1 mg) of coniferyl benzoate reference standard and transfer to a 5 ml volumetric flask and bring to volume with ethanol.

Standard solutions: Prepare five solutions, by the dilution of the standard stock solution of coniferyl benzoate with ethanol, in the concentration range of 0.05 to 0.8 mg/ml.

Sample preparation

Accurately weigh about 5 g (\pm 0.1 mg) (w_s) of the previously crushed Benzoe tonkinensis sample and solubilize in 20 ml of ethanol (96%).

Sonicate the mixture and filter. Re-extract the residue with a second

portion of 20 ml ethanol (96%), sonicate and filter. Combine the ethanol

extracts and evaporate the solvent under vacuum. Weigh accurately the

extracted resin (w_{ex}). Dilute the resin with ethanol to a final concentration (C_{resin}) of 1 mg/ml.

Procedure

Inject 10 μ l of each standard solution and record the peak areas. Plot a standard curve (concentration of coniferyl benzoate (mg/ml) (X-axis) vs. peak area of coniferyl benzoate (Y-axis)) and determine the slope (m)

and the linear coefficient (a).
Inject 10 µl of the sample and record the peak area.

Calculation

Calculate the content of coniferyl benzoate as follows:

$$\text{Coniferyl benzoate (w/w, \%)} = \left(\frac{A_{\text{ConBz}} - a}{m} \right) \times \frac{1}{C_{\text{resin}}} \times \frac{w_s}{w_{\text{ex}}} \times 100$$

where

A_{ConBz} is the peak area of coniferyl benzoate in the sample;

a is the linear coefficient of the standard curve;

m is the slope of the standard curve;

C_{resin} is the final concentration of the extracted resin diluted in ethanol;

w_{ex} is the weight of extracted resin with ethanol (g); and

w_s is the weight of sample of Benzoe tonkinensis (g).