Determination of residual solvents in annatto extracts (solvent-extracted bixin and norbixin)

(Tentative)

The Committee noted at the 77th meeting that the method for determination of residual solvent in the Combined Compendium of Food Additive Specifications, Volume 4 was not suitable for the determination of residual solvents in solvent-extracted bixin and norbixin. The Committee agreed that more results were needed to ensure that the method proposed below is a suitable substitute for the head-space gas chromatography method in the Specification and listed in Compendium of food additive specifications, Volume 4.

Chromatographic system

Detector:	Flame ionization detector (FID)
Column:	25% diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm I.D., 1.4 µm-film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas:	Helium
Flow rate:	205 kPa, 1.8 ml/min
Injector temperature:	260°
Detector temperature:	250°
Oven temperature:	Hold for 5min at 40°; then 40°to 92° at 4°/min; then Hold for 2 min at 92°, then 92° to 230° at 40°C/min

Head space sampler

Sample heating temperature:	60°
Sample heating period:	20 min
Syringe temperature:	100°
Transfer line temperature:	120°
Sample gas injection:	3.0 ml in split mode (25:1)

Stock standard solutions: Add 10 ml dimethylformamide to six 20 ml volumetric flasks. Accurately weigh, to within 0.01 mg, each flask. Pipet 250 µl each of chromatography grade methanol, ethanol, isopropanol, and ethyl acetate, and 150 µl each of acetone and hexane into each of the flask. Reweigh accurately and then fill the flask with dimethylformamide. Mix well.

Mixed standard solution A: Pipet each 3.0 ml of stock standard solution into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Mixed standard solution B: Pipet 4.0 ml solution A into a 10 ml volumetric flask and fill the flask with dimethylformamide.

Mixed standard solution C: Pipet 2.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Mixed standard solution D: Pipet 1.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Samples: Weigh accurately 0.2 g sample into a 20 ml head-space vial. Add 2.5 ml dimethylformamide and seal.

Standard solutions: Introduce 0.1 ml of the each standard mixture solution (A, B, C and D) into each 20 ml injection vial. Add 2.4 ml dimethylformamide and seal.

Standard curves

Place the four standard solutions in the sample tray on head-space gas chromatography. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical condition as described above. Measure the peak area for each solvent. Construct the standard curves by plotting the peak areas of each solvent against the concentrations of each solvent (mg/ml) in the standards solutions.

Procedure

Place the sample solution in the sample tray on head-space gas chromatograph. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical condition as described above. Measure the peak area for each solvent and obtain the concentration of each solvent from the standard curves. Calculate the concentration of each solvent as follows:

$$\text{Solvent (mg/kg)} = C \times 2.5/W \times 1000$$

Where:
C is the concentration of solvent (mg/ml).
W is weight of sample (g).