- **MERCURY,** *Mercury Limit Test,* Appendix IIIB
  Acceptance criteria: NMT 1 mg/kg

SPECIFIC TESTS

- **RESIDUE ON IGNITION (SULFATED ASH),** Appendix IIC
  Sample: 1 g
  Analysis: Proceed as directed, using a silica crucible and moistening the residue with 2 mL of nitric acid and 1 mL of sulfuric acid.
  Acceptance criteria: NMT 0.2%

Caramel

First Published: Prior to FCC 6

Caramel Color
INS: 150 CAS: [8028-89-5]
UNII: T9D99G2B1R [caramel]

DESCRIPTION

Caramel usually occurs as a dark brown to black liquid or solid. It is a complex mixture of compounds, some of which are in the form of colloidal aggregates. Caramel is manufactured by heating carbohydrates, either alone or in the presence of food-grade acids, alkalies, and/or salts. Caramel is produced from commercially available food-grade nutritive sweeteners consisting of fructose, dextrose (glucose), invert sugar, sucrose, malt syrup, molasses, and starch hydrolysates and fractions thereof. The acids that may be used are food-grade sulfuric, sulfurous, phosphoric, acetic, and citric acids; the alkalies are ammonium, sodium, potassium, and calcium hydroxides; and the salts are ammonium, sodium, and potassium carbonate, bicarbonate, phosphate (including mono- and dibasic), sulfate, and bisulfite. Food-grade antifoaming agents, such as polyglycerol esters of fatty acids, may be used as processing aids during its manufacture. Caramel is soluble in water.

Four distinct classes of Caramel can be distinguished by the reactants used in their manufacture and by specific identification tests:

Class I (Plain Caramel, Caustic Caramel): Prepared by heating carbohydrates with or without acids or alkalis; no ammonium or sulfite compounds are used.

Class II (Caustic Sulfite Caramel): Prepared by heating carbohydrates with or without acids or alkalis in the presence of sulfite compounds; no ammonium compounds are used.

Class III (Ammonia Caramel): Prepared by heating carbohydrates with or without acids or alkalis in the presence of ammonium compounds; no sulfite compounds are used.

Class IV (Sulfite Ammonia Caramel): Prepared by heating carbohydrates with or without acids or alkalis in the presence of both sulfite and ammonium compounds.

All of these Caramels shall meet the criteria established for Caramel in this monograph.

Function: Color

Packaging and Storage: Store in well-closed containers and avoid exposure to excessive heating and, for solid products, excessive humidity.

IMPURITIES

Inorganic Impurities

- **ARSENIC,** *Arsenic Limit Test,* Appendix IIIB
  Sample solution: Prepare as directed for organic compounds.
  Control: 1 µg As (1.0 mL of *Standard Arsenic Solution*)
  Acceptance criteria: NMT 1 mg/kg
- **LEAD**

  [NOTE—Use reagent-grade chemicals with as low a lead content as is practicable as well as high-purity water and gases. Before use, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid. Then rinse them thoroughly with high-purity water, preferably obtained from a mixed-bed, strong-acid, strong-base ion-exchange cartridge capable of producing water with an electrical resistivity of 12 to 15 megohms.]

  Lead nitrate solution: 100 µg Pb/mL, prepared by transferring 159.8 mg of ACS reagent-grade lead nitrate, $Pb(NO_3)_2$, into a 1000-mL volumetric flask, adding 100 mL of water containing 1 mL of nitric acid to dissolve the lead nitrate, diluting to volume with water, and mixing. [NOTE—Prepare and store this solution in glass containers that are free from lead salts.]

  Standard stock solution: 10 µg Pb/mL prepared by transferring 50.0 mL of *Lead nitrate solution* into a 500-mL volumetric flask containing 50 mL of water, adding 5 mL of nitric acid, diluting to volume with water, and mixing. [NOTE—Prepare on the day of use.]

  Standard solutions: 0.20, 0.50, 1.00 and 2.00 µg Pb/mL, prepared by pipeting 2, 5, 10, and 20 mL of *Standard stock solution* into separate 100-mL volumetric flasks, adding 1 mL of nitric acid to each, diluting to volume with water, and mixing.

  Sample solution: Transfer 25 g of sample into an ashing vessel. [NOTE—Suitable ashing vessels have approximately a 100-mL capacity and are flat-bottomed platinum crucibles or dishes, Vycor or quartz tall-form beakers, or Vycor evaporating dishes (Corning Glass Works No. 13180, or equivalent). Discard Vycor vessels when the inner surfaces become etched.] Dry the sample overnight at 120° in a forced draft oven. [NOTE—The sample must be absolutely dry to prevent flowing or spattering in the furnace.] Place the sample in a furnace equipped with a pyrometer to control the temperature over a range of 260° to 600°, with a variation of less than 10° and set the temperature at 250°. Slowly, in 50° increments, raise the temperature to 350°, and hold at this temperature until smoking ceases. Increase the temperature to 500° in approximately 75° increments; the sample must not ignite. Ash for 16 h or overnight at 500°. Remove the sample from the furnace and allow it to cool. The ash should be white and essentially carbon free. If the ash still contains excess carbon particles (i.e., the ash is gray rather than white), proceed as follows: Wet the

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ash with a minimal amount of water followed by the dropwise addition of 0.5 to 3 mL of nitric acid. Dry on a hot plate. Transfer the ash to a furnace set at 250°, slowly increase the temperature to 500°, and continue heating for 1 to 2 h. Repeat the nitric acid treatment and ashing, if necessary, to obtain a carbon-free residue. [NOTE—Local overheating or deflagration may result if the sample still contains much intermingled carbon and especially if much potassium is present in the ash.]

Dissolve the residue in 5 mL of 1 N nitric acid, warming on a steam bath or hot plate for 2 to 3 min to aid solution. Filter, if necessary, and decant through S&S 589 Black Ribbon paper, or equivalent, into a 50-mL volumetric flask. Repeat with two 5-mL portions of 1 N nitric acid, filter, and add the washings to the original filtrate. Dilute to volume with 1 N nitric acid and mix to prepare the *Sample solution*.

Reagent blanks: Prepare duplicate blanks for each *Standard solution* and for the *Sample solution*, including any additional water and nitric acid if used for sample ashing. [NOTE—Do not ash nitric acid in a furnace because the lead contaminant will be lost.] Evaporate nitric acid to dryness in an ashing vessel on a steam bath or hot plate. Dissolve the residue in 5 mL of 1 N nitric acid, warming on a steam bath or hot plate for 2 to 3 min to aid solution. Filter, if necessary, and decant through S&S 589 Black Ribbon paper, or equivalent, into a 50-mL volumetric flask. Repeat with two 5-mL portions of 1 N nitric acid, filter, and add the washings to the original filtrate. Dilute to volume with 1 N nitric acid, and mix to prepare each reagent blank.

[NOTE—Complete the sample preparation (above) and the analysis (below) on the same day.]

Aqueous butyl acetate: Use spectral-grade butyl acetate and saturate it with water.

APDC solution: 20 mg/mL of APDC (ammonium 1-pyrrolidinedithiocarbamate, Aldrich Chemical, or equivalent) made to 100 mL. Remove insoluble free acid and other impurities normally present by two to three extractions with 10-mL portions of *Aqueous butyl acetate*.

Citric acid solution, lead-free: Dissolve 10 g of citric acid in 30 mL of water. While stirring, slowly add ammonium hydroxide until the pH is between 8.0 and 8.5, using short-range pH paper as an external indicator. Transfer the solution into a separatory funnel, and extract with 10-mL portions of *Dithizone Extraction Solution* (see *Lead Limit Test,* Appendix IIIB), until the dithizone solution retains its green color or remains unchanged. Drain the final dithizone layer, plus about 1 mL of the aqueous layer, into a beaker and, while stirring, slowly add 1:1 nitric acid until the pH is between 3.5 and 4, again using short-range pH paper as an external indicator. Transfer this solution into a 100-mL volumetric flask (through a filter, if necessary), dilute to volume with water, and mix thoroughly.

Test solutions: Pipet 20 mL each of the *Standard solutions*, the *Sample solution*, and the appropriate *Reagent blanks* into separate 60-mL separatory funnels. Treat each solution as follows: Add 4 mL of *Citric acid solution, lead-free* and 2 to 3 drops of bromocresol green TS. The solution should be yellow. Adjust the pH to about 5.4, using ammonium hydroxide initially and then ammonium hydroxide diluted with 4 volumes of water in the vicinity of the color change (the first permanent appearance of light blue). Add 4 mL of *APDC solution*, stopper, and shake the funnel for 30 to 60 s. Pipet 5.0 mL of *Aqueous butyl acetate*, stopper the funnel, and shake the funnel vigorously for 30 to 60 s. Let stand until the layers separate clearly, drain, and discard the lower aqueous phase. If an emulsion forms or the solvent layer is cloudy, drain the solvent layer into a 15-mL centrifuge tube, cover the tube with aluminum foil or Parafilm (or equivalent), and centrifuge it for about 1 min at 2000 rpm.

Analysis: Use an atomic absorption spectrophotometer equipped with a 4-in., single-slot burner head. Set the instrument to previously determined optimum conditions for organic solvent aspiration (3 to 5 mL/min) and at a wavelength of 283.3 nm. Use an air–acetylene flame adjusted for maximum lead absorption with a fuel-lean flame. Aspirate each of the *Test solutions*, flushing with water and then with *Aqueous butyl acetate* between measurements and record their absorbance. Correct the absorbances of the *Test solutions* prepared from the *Standard solutions*, and the *Sample solution*, with the readings of the *Test solutions* prepared from the *Reagent blanks*. Prepare a standard curve by plotting the absorbance of each of the *Test solutions* prepared from the *Standard solutions* against their concentrations (µg Pb/mL). (These concentrations, in butyl acetate, are four times those in the aqueous standard.) From the standard curve, determine the concentration, C, (µg/mL), of the *Sample solution*. Calculate the quantity, in mg/kg, of lead in the sample by the formula:

$$\text{Result} = 12.5 \times C/W$$

W = weight of the sample taken (g)

Acceptance criteria: NMT 2 mg/kg

• **MERCURY,** *Mercury Limit Test, Method I,* Appendix IIIB

Standard preparation: Prepare as directed transferring 1.0 mL of the stock solution (1 µg Hg) into a 50-mL beaker rather than the specified 2.0 mL.

Sample preparation: Transfer 5 g of sample into a 250-mL Erlenmeyer flask and add 5 mL of sulfuric acid and a few glass beads. Digest the mixture at a temperature not exceeding 120° until charring begins, preferably using a hot plate in a fume hood. [NOTE—Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 mL.] After the acid has initially decomposed the sample, cautiously add, dropwise, hydrogen peroxide (30%), allowing the reaction to subside and reheating the sample between drops. Add the first few drops very slowly with sufficient mixing to prevent a rapid reaction and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on

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the walls or bottom of the flask during digestion. [NOTE—Maintain oxidizing conditions at all times during the digestion by adding small quantities of the peroxide whenever the mixture turns brown or darkens.] Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250° to 300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool the flask, cautiously add 10 mL of water, heat again to strong fuming, and cool. Cautiously add 10 mL of water, mix, wash the sides of the flask with a few milliliters of water, and dilute to 35 mL. Add 1 mL of a 1:25 solution of potassium permanganate, and mix.

Analysis: Continue as directed in the *Procedure*, using a suitable atomic absorption spectrophotometer.

Acceptance criteria: Any absorbance produced by the *Sample preparation* is not more than half that produced by *the Standard preparation*, indicating NMT 0.1 mg/kg.

• **SULFUR DIOXIDE,** *Sulfur Dioxide Determination,* Appendix X

Sample: 0.5 g

Analysis: Determine as directed, and calculate on an equivalent color basis expressed in terms of a caramel having a *Color Intensity* of 0.1 a.u. (absorbance unit).

Acceptance criteria: NMT 0.2%, calculated on the equivalent color basis

Organic Impurities

• **4-METHYLIMIDAZOLE**

4-Methylimidazole stock solution: Purify reagent-grade 4-methylimidazole (Aldrich, or equivalent) by redistillation (b.p. 92° to 93°, 0.05 mm Hg). Transfer 50 mg of the distillate, into a 50-mL volumetric flask, dilute to volume with tetrahydrofuran (acetone is also acceptable), and mix thoroughly. Store in a refrigerator until ready for use.

Standard solutions: 100, 150, 200, 250, 300, 350, 400, and 500 µg/mL prepared by pipetting, respectively 1.0-, 1.5-, 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, and 5.0-mL portions of *4-Methylimidazole stock solution* into separate 10-mL volumetric flasks, diluting each to volume with the same solvent used to prepare the stock solution, and mixing. Store the solutions in a refrigerator; use them within 1 month.

Sample solution: Transfer 10.0 g of sample into a 250-mL polypropylene beaker, and mix thoroughly with 5.0 g of 3 N sodium hydroxide; the pH of the mixture should exceed 12. Add 20.0 g of chromatographic siliceous earth (Johns-Manville Celite 545, or equivalent) to the beaker and thoroughly mix with a wide-blade, stainless steel spatula until a homogeneous, semidry mixture is obtained. Homogeneity is obtained when the color is uniform and no dark clumps are seen. Place a plug of fine glass wool in the base of a 300-× 22-mm (id) chromatography tube having a Teflon stopcock. Quantitatively transfer the mixture into the column. The column bed, approximately 150 mm tall, should be of uniform consistency, yet open enough to allow elution to occur readily. Place a plug of glass wool on top of the column, and then allow the column to fall a short distance vertically to help settle the contents.

Rinse the sample beaker with methylene chloride and pour the contents into the column with the stopcock open. Allow the methylene chloride to pass down the column until it reaches the stopcock. Close the stopcock and allow the methylene chloride to remain in contact with the column bed for 5 min. Open the stopcock and pass methylene chloride through the column at a rate of 5 mL/min. Collect 200 mL of eluate in a 300-mL round-bottom flask. While maintaining the flask at 35° in a water bath, remove the bulk of the solvent from the eluate by rotary vacuum evaporation (350 to 390 mm Hg). Reduce the volume to about 1 mL. During the concentration step, watch the flask carefully to ensure that no loss of sample occurs by bumping. Use a disposable Pasteur pipet to quantitatively transfer the extract residue to a 5-mL volumetric flask, rinsing the flask several times with small (approximately 0.7 mL) portions of the same solvent used to prepare the original solutions (tetrahydrofuran or acetone); transfer the rinsings to the volumetric flask until the 5-mL dilution mark is reached. Mix thoroughly.

Chromatographic system, Appendix IIA

Mode: Gas chromatography

Detector: Hydrogen flame-ionization

Column: 1-m × 4-mm (id) silanized glass column, or equivalent, packed with 90- to 100-mesh Anakrom ABS, or equivalent, containing 7.5% Carbowax 20M and 2% potassium hydroxide, or equivalent

Column temperature: 190°

Injection port temperature: 200°

Detector temperature: 250°

Carrier gas: Nitrogen

Flow rate: 50 mL/min

Injection volume: 5.0 µL

Analysis: [NOTE—Preferably, use an autosampler to inject the *Standard solutions*. If using manual injections, avoid fractionation in the syringe needle, and ensure that 5.0 µL is injected by using the solvent-flush technique with the solvent used to prepare the *Standard solutions*.]

Inject the *Standard solutions* into the chromatograph and obtain the chromatograms. From each chromatogram, obtain the corrected peak area. If not using an integrator, calculate the corrected peak area by multiplying the peak height (mm) by the peak width at one-half height (mm), and by the proper attenuation and range factors, depending on the particular apparatus and operating parameters used. Plot each corrected peak area versus its respective concentration of 4-methylimidazole to obtain the standard curve. In the same manner, chromatograph a 5.0-µL portion of the *Sample solution*, calculate the peak area corresponding to any 4-methylimidazole contained in the sample, and, by reference to the standard curve, obtain the content of the 4-methylimidazole in the sample. Calculate the percent 4-methylimidazole on an equivalent color basis

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expressed in terms of a Caramel having a *Color Intensity* of 0.1 a.u.

Acceptance criteria: NMT 0.025%, calculated on the equivalent color basis

SPECIFIC TESTS

- **AMMONIACAL NITROGEN**

Sample: 2 g

Analysis: Transfer 25.0 mL of 0.1 N sulfuric acid into a 500-mL receiving flask. Connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, making certain that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask. Transfer the *Sample* into an 800-mL long-neck Kjeldahl digestion flask and add 2 g of carbonate-free magnesium oxide, 200 mL of water, and several boiling chips to the flask. Swirl the digestion flask to mix the contents, and quickly connect it to the distillation apparatus. Heat the contents of the flask to boiling and collect about 100 mL of distillate in the receiving flask. Wash the tip of the delivery tube with a few milliliters of water, collecting the washings in the receiving flask. Add 4 or 5 drops of methyl red TS, titrate with 0.1 N sodium hydroxide, and record the volume (mL) as S. Conduct a blank determination (see *General Provisions*) and record the mL of 0.1 N sodium hydroxide required as B. Calculate the percent *Ammoniacal Nitrogen* (on an equivalent color basis) by the formula:

$$\text{Result} = [(B - S) \times W_N \times 100/W] \times F/A_{610}$$

W_N = the mEq weight of nitrogen for 0.1 N sodium hydroxide, 0.0014
W = weight of sample taken (g)
F = the basis of color equivalency, 0.1
A_{610} = absorbance of the 0.1% solution prepared for the determination of *Color intensity* (below)

[NOTE—The above formula gives the result on an equivalent color basis that permits expression in terms of a caramel having a color intensity standardized to 1 a.u.]

Acceptance criteria: NMT 0.6%, calculated on the equivalent color basis

- **COLOR INTENSITY**

Sample solution: 1 mg/mL (0.1%) [NOTE—Centrifuge if the solution is cloudy.]

Analysis: Using a suitable spectrophotometer and a 1-cm cell, determine the absorbance (A_{610}) of the clear *Sample solution* at 610 nm; use water as the blank. Calculate the *Color intensity* by the formula:

$$\text{Result} = (A_{610} \times 100)/S$$

S = the percent *Total solids* (see test below)

Acceptance criteria: Between 0.01 and 0.6 absorbance units (a.u.)

- **TOTAL NITROGEN,** *Nitrogen Determination, Method II,* Appendix IIIC

Analysis: Determine as directed and calculate on an equivalent color basis expressed in terms of a caramel having a *Color Intensity* of 0.1 a.u.

Acceptance criteria: NMT 3.3%, calculated on the equivalent color basis

- **TOTAL SOLIDS**

Liquid samples

Sample: 1.5-2.0 g

Analysis: Mix the *Sample* with 30.0 g of fine quartz sand that passes a No. 40, but not a No. 60 sieve, and that has been digested with hydrochloric acid, washed acid-free, dried, and ignited. Dry the mixture to constant weight at 60° under reduced pressure (50 mm Hg). Record the final weight of the sand plus the sample solids and calculate the percent solids as follows:

$$\text{Result} = [(W_F - W_S)/W_C] \times 100$$

W_F = weight of the sand and sample solids (g)
W_S = weight of the prepared sand taken (g)
W_C = weight of the sample taken (g)

Use the calculated percent *Total solids* in the calculation for *Color intensity* (see above).

Solid samples (powdered or granular)

Analysis: Determine as directed under *Loss on Drying,* Appendix IIC, drying a sample at 60° under reduced pressure (50 mm Hg) to constant weight. Calculate the percent solids as follows:

$$\text{Result} = [(W_D - W_B)/(W_S - W_B)] \times 100$$

W_D = weight of the bottle and sample after drying (g)
W_B = weight of the empty bottle (g)
W_S = weight of the bottle and sample before drying (g)

Use the calculated percent *Total solids* in the calculation for *Color intensity* (see above).

- **TOTAL SULFUR**

Sample: 5 g when the expected amount of sulfur is 2.5% or less; or 1 g when the expected amount of sulfur is greater than 2.5%

Analysis: Into the largest casserole available that fits in an electric muffle furnace, place: 1 to 3 g of magnesium oxide or an equivalent quantity of magnesium nitrate, hexahydrate (6.4 to 19.2 g); 1 g of powdered sucrose; and 50 mL of nitric acid. Transfer the *Sample* into the casserole. Place the same quantities of reagents in another casserole for the blank and carry through the following procedure for both the *Sample* and the blank: Evaporate the casserole contents on a steam bath to the consistency of paste. Place the casserole in a cold electric muffle furnace, gradually heat to 525°, and hold at that temperature until all nitrogen dioxide fumes are driven off. Cool the casserole, add 100 mL of water (the sample should dissolve), and neutralize to pH 7 with hydrochloric acid, using short-range pH indicator paper as an external indicator. Add an additional 2 mL of hydrochloric acid, filter the solution

Monographs

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into a suitable beaker, heat to boiling, and while stirring, slowly add 20 mL of barium chloride TS to the hot solution. Boil the contents of the beaker for 5 min, and allow it to stand overnight. Filter the contents of the beaker through a tight, ashless filter paper and quantitatively transfer the precipitate to the paper. Thoroughly wash the paper and the precipitate with hot water and transfer the paper to a tared crucible previously ignited for 1 h at 800° in a muffle furnace. Dry the paper in the crucible for 1 h at 105°. Then carefully char it, with free access to air, at low heat over a burner. Gradually increase the heat to burn away the paper and ignite the crucible and contents for 1 h at 800°. Cool and weigh it and calculate the percent sulfur by the formula:

$$\text{Result} = [(W_S - W_B)/S] \times 13.74 \times F/A_{610}$$

W_S = weight of the ignited residue of barium sulfate from the sample determination (g)
W_B = weight of the ignited residue from the blank determination (g)
S = weight of the sample taken (g)
F = the basis of color equivalency, 0.1
A_{610} = absorbance of the 0.1% solution prepared for the determination of *Color intensity* (above)

[NOTE—The above formula gives the result on an equivalent color basis that permits expression in terms of a caramel having a color intensity standardized to 1 a.u.]

Acceptance criteria: NMT 3.5%, calculated on the equivalent color basis

ADDITIONAL INFORMATION

- **IDENTIFICATION OF CLASSES**

[NOTE—The four classes of Caramel may be distinguished from each other by the methods below.]

Class I: Not more than 50% of the color is bound by DEAE (diethylaminoethyl) cellulose, and not more than 50% of the color is bound by phosphoryl cellulose.

Class II: More than 50% of the color is bound by DEAE cellulose and it exhibits an *Absorbance Ratio* (see below) of more than 50.

Class III: Not more than 50% of the color is bound by DEAE cellulose, and more than 50% of the color is bound by phosphoryl cellulose.

Class IV: More than 50% of the color is bound by DEAE cellulose and it exhibits an *Absorbance Ratio* (see below) of not more than 50.

- **IDENTIFICATION TESTS FOR CLASSES**

Tests

- ABSORBANCE RATIO

Sample solution: 1 mg/mL [NOTE—Any cloudiness appearing can be eliminated by centrifuging the solution.]

Dilute sample solution: 50 µg/mL: made from *Sample solution*

Analysis: Use a spectrophotometer equipped with a monochromator to provide a band width of 2 nm or less and of such quality that the stray-light characteristic is 0.5% or less. With water as a reference, measure the absorbance of the *Sample solution* solution in a 1-cm cell at 560 nm and that of the *Dilute sample solution* at 280 nm. Calculate the *Absorbance Ratio* of the sample by the formula:

$$\text{Result} = (A_{280} \times 20)/A_{560}$$

A_{280} = absorbance at 280 nm for the *Dilute sample solution*
A_{560} = absorbance at 560 nm for the *Sample solution*
20 = dilution factor

- COLOR BOUND BY DEAE CELLULOSE

[NOTE—*Color Bound by DEAE Cellulose* is defined here as the percent decrease in absorbance of a caramel solution at 560 nm after treatment with DEAE cellulose.]

DEAE cellulose: Use material with a capacity of 1.0 mEq/g. *DEAE cellulose* of higher or lower capacities may be used in proportionately higher or lower quantities.

Sample solution: Prepare a *Sample solution* of approximately 0.5 absorbance unit at 560 nm by transferring an appropriate amount of sample into a 100-mL volumetric flask with the aid of 0.025 N hydrochloric acid. Dilute to volume with 0.025 N hydrochloric acid, and centrifuge or filter if the solution is cloudy.

Supernatant: To a 20-mL aliquot of the *Sample solution*, add 140 mg of *DEAE cellulose*, mix thoroughly for several minutes, centrifuge or filter, and collect the clear supernatant liquid.

Analysis: Using a suitable spectrophotometer previously standardized with 0.025 N hydrochloric acid, measure the absorbance of the *Sample solution* and of the *Supernatant* in a 1-cm cell at 560 nm. Calculate the percent of color bound by *DEAE cellulose* by the formula:

$$\text{Result} = 100[(X_1 - X_2)/X_1]$$

X_1 = absorbance of the *Sample solution* at 560 nm
X_2 = absorbance of the *Supernatant* at 560 nm

- COLOR BOUND BY PHOSPHORYL CELLULOSE

[NOTE—*Color Bound by Phosphoryl Cellulose* is defined here as the percent decrease in absorbance of a caramel solution at 560 nm after treatment with phosphoryl cellulose.]

Phosphoryl cellulose: Use material with a capacity of 1.2 mEq/g. *Phosphoryl cellulose* (cellulose phosphate) of higher or lower capacities may be used in proportionately higher or lower quantities.

Sample solution: Transfer 200 to 300 mg of sample into a 100-mL volumetric flask, dilute to volume with 0.025 N hydrochloric acid; centrifuge or filter if the solution is cloudy.

Supernatant: To a 40-mL aliquot of the *Sample solution*, add 1.42 g of *Phosphoryl cellulose*, mix

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thoroughly for several minutes, centrifuge or filter, and collect the clear supernatant liquid.

Analysis: Using a suitable spectrophotometer previously standardized with 0.025 N hydrochloric acid, measure the absorbance of the *Sample solution* and of the *Supernatant* in a 1-cm cell at 560 nm. Calculate the percent of color bound by *Phosphoryl cellulose* by the formula:

$$\text{Result} = 100[(X_1 - X_2)/X_1]$$

X_1 = absorbance of the *Sample solution* at 560 nm

X_2 = absorbance of the *Supernatant* at 560 nm

- **2-ACETYL-4(5)-TETRAHYDROXYBUTYLIMIDAZOLE (THI)**

[NOTE—Class III (Ammonia Caramel) is the only class of caramel color found to contain THI. Because some countries have a THI limit of 25 mg/kg on an equivalent color basis (on the basis of a product with *Color intensity* standardized to 0.1 absorbance units), the following method for determining THI is provided.]

2,4-Dinitrophenylhydrazine-hydrochloride (DNPH-HCl): Add 5 g of reagent-grade 2,4-dinitrophenylhydrazine (DNPH) to 10 mL of hydrochloric acid contained in a 100-mL Erlenmeyer flask, and gently shake the latter until the free base (red) is converted to the hydrochloride (yellow). Add 100 mL of ethanol and heat the mixture on a steam bath until all of the solids have dissolved. Cool to room temperature and, after the solution has crystallized, filter off the hydrochloride. Wash the hydrochloride with ether, dry it at room temperature, and store it in a desiccator. Upon storage, the hydrochloride slowly converts to the free base. The latter can be removed by washing with purified (peroxide-free) dimethoxyethane.

DNPH-HCl reagent: Mix 0.5 g of *DNPH–HCl* with 15 mL of 5% methanol in dimethoxyethane for 30 min. Store in a refrigerator at 4°. When properly prepared and stored, this reagent is stable for at least 3 months.

THI-DNPH standard: Add 0.5 g of *DNPH–HCl* to 1 mL of hydrochloric acid, followed by 10 mL of ethyl alcohol, and heat on a steam bath until the hydrochloride dissolves. Add 100 mg of 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI) to the hot solution. Crystallization begins in a few minutes. Filter off the THI-DNPH when the suspension reaches room temperature. Recrystallize the THI-DNPH with a hydrochloric acid-ethyl alcohol mixture (1 drop of hydrochloric acid per 5 mL of ethyl alcohol). The yield is 70% to 80% based on the THI used. When stored in the refrigerator, the *THI–DNPH standard* is stable for at least 1 year.

Stock THI-DNPH solution: Dissolve 10 mg of *THI–DNPH standard* in a 100-mL volumetric flask and dilute to volume with absolute *Carbonyl-free methanol* (see below). Dilute a portion of this solution tenfold with the methanol. The THI concentration (mg/L) of the *Stock THI–DNPH solution* is 0.47 times that of *THI–DNPH standard*. When stored in the refrigerator, the *Stock THI–DNPH solution* is stable for at least 20 weeks.

Cation-exchange resin (Strong): Dowex 50 AG × 8, proton form, 100- to 200-mesh

Cation-exchange resin (Weak): Amberlite CG AG 50 I, proton form, 100- to 200-mesh [NOTE—Sediment two or three times before use.]

Carbonyl-free methanol: Add 5 g of Girard's Reagent P (Aldrich, or equivalent) and 0.2 mL of hydrochloric acid to 500 mL of methanol and reflux for 2 h. Distill the refluxed methanol through a short Vigreux column, and store in tightly closed bottles.

Purified dimethoxyethane: Distill dimethoxyethane from DNPH in the presence of acid and redistill it from sodium hydroxide. Immediately before use, pass it through a column of neutral alumina to remove peroxides.

Combination column

Dropping funnel: 100-mL, equipped with a Teflon stopcock and fitted with a 14.5-mm standard ground-glass joint, as the solvent reservoir

Upper column: Glass, 150 × 12.5 mm (id), filling height: max 9 cm and bed height: 50 to 60 mm; or 200 × 10 mm (id), filling height: max 14 cm and bed height 80 to 90 mm, equipped with a 1-mm (id) capillary outlet and fitted with 14.5-mm standard ground-glass joints

Lower column: Glass, 175 mm × 10 mm (id), bed height 60 mm, equipped with a 1-mm (id) capillary outlet and a Teflon stopcock and fitted with a 14.5-mm standard ground-glass joint

Assembly: Fill the *Upper column* with *Cation-exchange resin (Weak)*. Fill the *Lower column* with *Cation-exchange resin (Strong)*. Connect the *Dropping funnel* and the two columns, one fitted above the other.

Sample solution: Dissolve 200 to 250 mg of sample in 3 mL of water. Quantitatively transfer the solution to the upper part of the *Combination column*. Elute with water until a total of about 100 mL of water has passed through the column. Disconnect the *Upper column* and elute the *Lower column* with 0.5 N hydrochloric acid. Discard the first 10.0 mL of eluate and subsequently collect a volume of 35 mL. Concentrate the solution to dryness at 40° (15 mm Hg). Then, dissolve the syrupy residue in 250 µL of *Carbonyl-free methanol* and add 250 µL of *DNPH–HCl reagent*. Transfer the reaction mixture (*Sample solution*) to a septum-capped vial, and store the vial for 5 h at room temperature.

Mobile phase: 50:50 (v/v) methanol:0.1 M phosphoric acid

THI–DNPH standard solutions: Pipet 1, 2, and 5 mL of the *Stock THI–DNPH solution* into separate 10-mL volumetric flasks and dilute to volume with absolute *Carbonyl-free methanol*.

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 385 nm

Column: 250-mm × 4-mm (id), 10-1m LiChrosorb RP-8 HPLC column (Alltech Associates, Inc., or equivalent)

Monographs

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Injection volume: 5 µL
Flow rate: 2 mL/min
Analysis: Prepare a standard curve by injecting the *Stock THI–DNPH solution*, and the serially diluted *THI–DNPH standard solutions*, recording the chromatograms, and measuring the peak areas for THI–DNPH. Inject the *Sample solution* and measure the peak response. [NOTE—THI-DNPH elutes at about 6.3±0.1 min.] Calculate the amount of THI in the sample from the standard curve. [NOTE—For THI limits greater than 25 mg/kg, prepare a series of *THI–DNPH standard solutions* in a range encompassing the expected THI concentration in the sample.]

Caraway Oil

First Published: Prior to FCC 6

CAS: [8000-42-8]

UNII: C2J9B08Q3I [caraway oil]

DESCRIPTION

Caraway Oil occurs as a colorless to pale yellow liquid with the characteristic odor and taste of caraway. It is a volatile oil distilled from the dried, ripe fruit of *Carum carvi* L. (Fam. Umbelliferae).

Function: Flavoring agent

Packaging and Storage: Store in full, tight containers in a cool place protected from light.

IDENTIFICATION

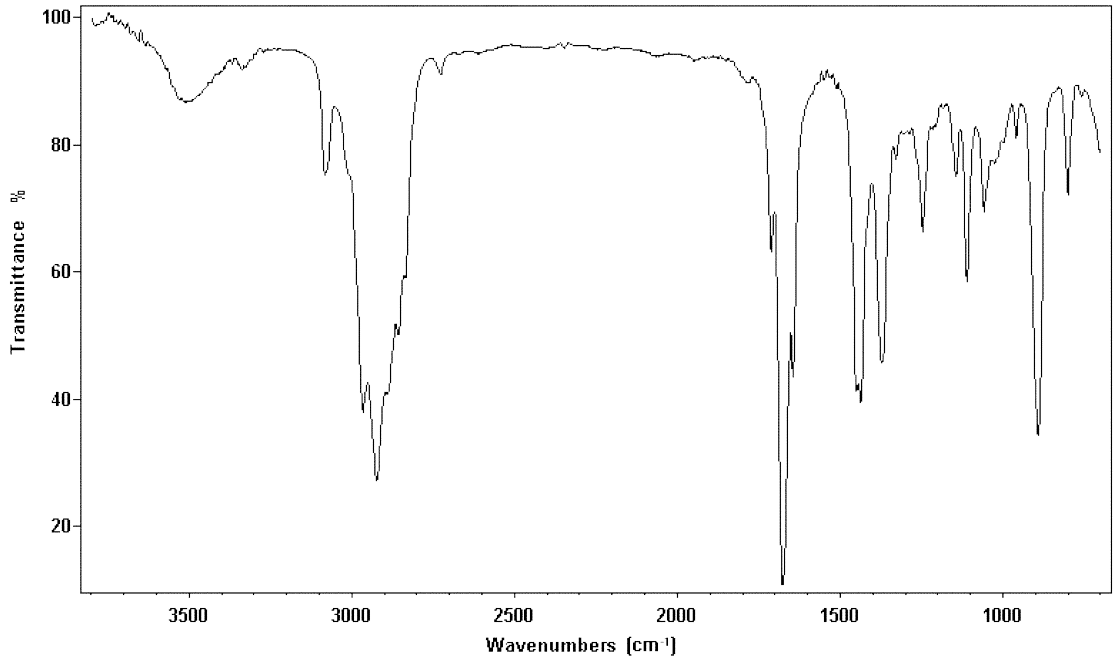
- **INFRARED SPECTRA,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.

ASSAY

- **KETONES,** *Aldehydes and Ketones, Neutral Sulfite Method,* Appendix VI
  Acceptance criteria: NLT 50.0%, by volume, of ketones as carvone

SPECIFIC TESTS

- **ANGULAR ROTATION,** *Optical (Specific) Rotation,* Appendix IIB
  Acceptance criteria: Between +70° and +80°
- **REFRACTIVE INDEX,** Appendix IIB
  [NOTE—Use an Abbé or other refractometer of equal or greater accuracy.]
  Acceptance criteria: Between 1.484 and 1.488 at 20°
- **SOLUBILITY IN ALCOHOL,** Appendix VI
  Acceptance criteria: One mL of the sample dissolves in 8 mL of 80% alcohol.
- **SPECIFIC GRAVITY:** Determine by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.900 and 0.910



Caraway Oil

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