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Add the following:

^Conjugated Linoleic Acids—Triglycerides

DEFINITION

Conjugated Linoleic Acids—Triglycerides are derived from plant seed oils such as safflower oil. They consist of NLT 77% conjugated linoleic acids—triglycerides and NLT 78% of the total fatty acids are conjugated linoleic acids (CLAs). They contain a 1:1 ratio of the active conjugated linoleic isomers (C18:2 *cis*-9, *trans*-11 and C18:2 *trans*-10, *cis*-12). Tocopherol may be added as an antioxidant.

IDENTIFICATION

- **A.** It meets the requirements in the test for *Fats and Fixed Oils, Fatty Acid Composition* under *Specific Tests*.
- **B.** It meets the requirements in the test for *Content of Conjugated Linoleic Acids—Tri-, Di-, Monoglycerides and Polymerized Triglycerides*.

COMPOSITION

- **CONTENT OF CONJUGATED LINOLEIC ACIDS—TRI-, DI-, MONOGLYCERIDES AND POLYMERIZED TRIGLYCERIDES**

Mobile phase: [Tetrahydrofuran](#)

System suitability solution: 0.5 mg/mL of monodocosahexaenoin, 0.3 mg/mL of didocosahexaenoin, and 0.2 mg/mL of tridocosahexaenoin¹ in *Mobile phase*

Standard solution: Transfer about 50 mg of [USP Conjugated Linoleic Acids—Triglycerides RS](#) to a 10-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume.

Sample solution: Transfer about 50 mg of Conjugated Linoleic Acids—Triglycerides to a 10-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: Differential refractometer

Columns: Three in series, 7.8-mm × 30-cm each; 5-μm packing [L21](#), with the following pore sizes:

Column 1: 50 nm

Column 2: 10 nm

Column 3: 5 nm

Column sequence: Injector—Column 1—Column 2—Column 3—Detector

Flow rate: 0.8 mL/min

Injection volume: 40 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin, *System suitability solution*

Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin, *Standard solution*

Chromatogram similarity: The chromatogram of the *Standard solution* is similar to the reference chromatogram provided with the lot of [USP Conjugated Linoleic Acids—Triglycerides RS](#) being used.

Analysis

Sample: *Sample solution*

Identify the peaks for polymerized triglycerides (peak or peaks that elute before the CLA-triglycerides peak), CLA-triglycerides, CLA-diglycerides, and CLA-monoglycerides in the *Sample solution* by comparing with those of the *Standard solution*.

Separately calculate the percentages of polymerized triglycerides, CLA-triglycerides, CLA-diglycerides, and CLA-monoglycerides in the portion of Conjugated Linoleic Acids—Triglycerides taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of polymerized triglycerides, CLA-triglycerides, CLA-diglycerides, or CLA-monoglycerides from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria

Polymerized triglycerides: NMT 3.0%

CLA-triglycerides: NLT 77.0%

CLA-diglycerides: NMT 23.0%

CLA-monoglycerides: NMT 1.0%

IMPURITIES

• LIMIT OF BENZO[a]PYRENE

[NOTE—An equivalent method such as the one described in ISO 22959 can be used instead.]

[CAUTION—Benzo[a]pyrene is a known carcinogen. Perform all work under an appropriate hood.]

Mobile phase: [Acetonitrile](#) and [water](#) (88:12)

Standard stock solution: 0.5 mg/mL of benzo[a]pyrene in [toluene](#). Store in the dark at 4°. Discard this solution after 6 months.

Standard solutions: Prepare four solutions having concentrations of 0.004, 0.02, 0.1, and 0.2 µg/mL of benzo[a]pyrene by diluting the *Standard stock solution* with [acetonitrile](#).

Extraction column: Solid-phase extraction (SPE),² freshly conditioned by washing the column with 5 mL of [dichloromethane](#) followed by 5 mL of [n-hexane](#).

Sample solution: Transfer about 0.5 g of Conjugated Linoleic Acids—Triglycerides to a 10-mL centrifuge tube. Add 3 mL of [n-hexane](#) to the tube and mix to dissolve the sample. Transfer this solution to the *Extraction column*. Rinse the sample tube with 2 mL of [n-hexane](#) and transfer to the *Extraction column*. Wash the *Extraction column* with 10 mL of [n-hexane](#). Elute the benzo[a]pyrene with 5 mL of [dichloromethane](#), and collect the eluate in a 10-mL centrifuge tube. Evaporate the eluate in a water bath at 35° under a gentle stream of nitrogen until the tube is completely dry. Dissolve the residue with about 1 mL of petroleum ether and transfer the solution quantitatively to a preweighed 2-mL vial. Evaporate the solution in the 2-mL vial until almost dry. Rinse the tube with 1 mL of petroleum ether and transfer the solution to the vial. Continue evaporating the solution until the vial is completely dry. Weigh the vial and determine the mass of the residue. The mass should be NMT 45 mg; otherwise repeat the *Sample solution* preparation steps. Cap the vial and store at 4°. Before use, dissolve the residue with a 300-µL mixture of [tetrahydrofuran](#) and [acetonitrile](#) (1:1). Pass the solution through a filter of 0.45-µm pore size.

Blank: Prepare as directed for the *Sample solution* but without the Conjugated Linoleic Acids—Triglycerides.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: Fluorometric

Excitation wavelength: 384 nm

Emission wavelength: 406 nm

Columns

Guard: 4.6-mm × 7.5-cm; 5-µm packing [L1](#)

Analytical: 4.6-mm × 25-cm; 5-µm packing L118

Flow rate: 1.0 mL/min

Injection volume: 10 µL. [NOTE—If an autosampler is used, the sample loop should be flushed with [acetonitrile](#) between injections.]

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Inject the *Standard solutions* and construct a four-point calibration curve using the peak response area versus concentration (ng/µL).

Separately inject the *Blank* and *Sample solution*, and determine the benzo[a]pyrene concentration (ng/µL) of each from the standard calibration curve.

Calculate the benzo[a]pyrene content, in µg/kg, in the portion of Conjugated Linoleic Acids—Triglycerides taken:

$$\text{Result} = (C - C_0) \times (V/M)$$

C = concentration of benzo[a]pyrene in the *Sample solution* as determined from the calibration curve (ng/µL)

C_0 = concentration of benzo[a]pyrene in the *Blank* as determined from the calibration curve (ng/µL)

V = volume of acetonitrile and tetrahydrofuran (1:1) added to the vial, 300 µL



Documents

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