

Status: Currently Official on 18-Feb-2025
 Official Date: Official as of 01-May-2023
 Document Type: Dietary Supplement Monographs
 DocId: GUID-538274D1-0E1E-44B6-9B50-FEF9E7465969_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M6483_03_01
 DOI Ref: yfb4v

© 2025 USPC
 Do not distribute

Conjugated Linoleic Acids–Free Fatty Acids

Change to read:

DEFINITION

▲ Conjugated Linoleic Acids–Free Fatty Acids are a mixture of free fatty acids derived from plant seed oils such as safflower oil. They contain NLT 78% of conjugated linoleic acids (CLAs) of different isomers, including CLA isomer *cis*-9,*trans*-11, CLA isomer *trans*-10,*cis*-12, CLA isomers *cis* *cis*, and CLA isomers *trans* *trans*. The ratio of the C18:2 *cis*-9,*trans*-11 and C18:2 *trans*-10,*cis*-12 isomers is 1:1. Suitable antioxidants in appropriate concentrations may be added.▲ (USP 1-May-2023)

IDENTIFICATION

• **A.** It meets the requirements in the test for *Fats and Fixed Oils, Fatty Acid Composition in Specific Tests*.

IMPURITIES

Change to read:

• **LIMIT OF BENZO[a]PYRENE**

[NOTE—An equivalent method such as the one described in ISO 22959^{▲1}▲ (USP 1-May-2023) can be used instead.]

[CAUTION—Benzo[a]pyrene is a known carcinogen. Carry out all work in 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 it after 6 months.

Standard solutions: Prepare four solutions with 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](#).

Sample solution: Transfer about 0.5 g of Conjugated Linoleic Acids–Free Fatty Acids 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 a freshly conditioned solid-phase extraction (SPE) column.² [NOTE—The SPE column is conditioned by washing the column first with 5 mL of [dichloromethane](#) and then with 5 mL of [n-hexane](#).] Rinse the sample tube with 2 mL of [n-hexane](#) and transfer to the SPE 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 pre-weighed 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 Conjugated Linoleic Acids–Free Fatty Acids.

Chromatographic system

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

Mode: LC

Detector: Fluorescence

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 [L1](#)

Flow rate: 1 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 4-point calibration curve using the peak area versus concentration (ng/µL). Inject each *Blank* and *Sample solution*, and determine its benzo[a]pyrene concentration (ng/µL) from the standard calibration curve.

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

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

C = concentration of benzo[a]pyrene obtained from the calibration curve for the oil sample (ng/μL)

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

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

M = mass of the oil sample (g)

Acceptance criteria: NMT 2 μg/kg

SPECIFIC TESTS

- **WATER DETERMINATION** (921), *Method I, Method Ia*: NMT 0.1%
- **FATS AND FIXED OILS** (401), *Procedures, Acid Value*: 195–204
- **FATS AND FIXED OILS** (401), *Procedures, Peroxide Value*: NMT 5.0

Change to read:

- **FATS AND FIXED OILS**, (401), *Procedures, Fatty Acid Composition*

Standard solution: Prepare as directed in the *Test solution*, except use 100 mg of [USP Conjugated Linoleic Acids-Free Fatty Acids RS](#).

Sample solution: Prepare as directed in the *Test solution*, except use 100 mg of Conjugated Linoleic Acids-Free Fatty Acids.

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for the fatty acid components are shown in [Table 1](#).]

Suitability requirements

Resolution: NLT $\Delta 1.1$ ▲ (USP 1-May-2023) between CLA isomer *cis*-9,*trans*-11 and CLA isomer *trans*-10,*cis*-12

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

Acceptance criteria: Conjugated Linoleic Acids-Free Fatty Acids exhibit the composition profile of fatty acids in [Table 1](#).

Table 1

Fatty Acid	Shorthand Notation	Relative Retention Time	Area Percentage
Palmitic acid	16:0	0.82	≤9
Stearic acid	18:0	0.92	≤5
Oleic acid	18:1	0.93	≤20
Linoleic acid	18:2	0.96	≤3
CLA	18:2	—	≥78
CLA isomer <i>cis</i> -9, <i>trans</i> -11	18:2	1.00	≥37.5
CLA isomer <i>trans</i> -10, <i>cis</i> -12	18:2	1.01	≥37.5
▲CLA isomers <i>cis cis</i>	18:2	1.01	≤3.0▲ (USP 1-May-2023)
CLA isomers <i>trans trans</i>	18:2	1.03	≤2.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a cool, dry place, and protect from light, heat, and air.
- **USP REFERENCE STANDARDS** (11).
[USP Conjugated Linoleic Acids-Free Fatty Acids RS](#)

¹ See www.iso.org/standard/41251.html.

² Chromspher PI 120Å PAH, HPLC column, 3.0-mm × 8-cm, 5-μm, Agilent, part number CP28159; www.agilent.com.

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.