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Add the following:

▲Annatto Seed Oil Tocotrienols

DEFINITION

Annatto Seed Oil Tocotrienols is the tocotrienol fraction derived from the oil of the seeds of the annatto tree (*Bixa orellana* L.). It contains NLT 70% of total tocotrienols calculated on the anhydrous basis. Of the total tocotrienols content, NLT 84% and NMT 92% is δ -tocotrienol and NLT 8% and NMT 16% is γ -tocotrienol. It contains NMT 1% of α -tocopherol on the anhydrous basis. It may contain added vegetable edible oils.

IDENTIFICATION

- **A.** The retention times of the δ -tocotrienol and γ -tocotrienol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

COMPOSITION

• CONTENT OF δ - AND γ -TOCOTRIENOLS

[NOTE—Use low-actinic glassware.]

Mobile phase: [1,4-Dioxane](#) and [hexane](#) (5:95)

Standard stock solution: 8 mg/mL of [USP Annatto Seed Tocotrienols Extract RS](#) in [tetrahydrofuran](#)

Standard solution 1: Evaporate an aliquot of 2.0 mL of the *Standard stock solution* to dryness and reconstitute the residue in 100.0 mL of the *Mobile phase*.

Standard solution 2: Evaporate an aliquot of 2.0 mL of the *Standard stock solution* to dryness and reconstitute the residue in 25.0 mL of the *Mobile phase*.

Sample stock solution: 8 mg/mL of Annatto Seed Oil Tocotrienols in [tetrahydrofuran](#)

Sample solution 1: Evaporate an aliquot of 2.0 mL of the *Sample stock solution* to dryness and reconstitute the residue in 100.0 mL of the *Mobile phase*.

Sample solution 2: Evaporate an aliquot of 2.0 mL of the *Sample stock solution* to dryness and reconstitute the residue in 25.0 mL of the *Mobile phase*.

Chromatographic system

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

Mode: LC

Detector: UV 290 nm

Column: 4-mm $\times$ 15-cm; 3- μ m packing [L20](#)

Autosampler temperature: 15°

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution 1* and *Standard solution 2*

[NOTE—The relative retention times of the γ -tocotrienol and δ -tocotrienol peaks are 1.0 and 1.5, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for both δ -tocotrienol (*Standard solution 1*) and γ -tocotrienol (*Standard solution 2*)

Relative standard deviation: NMT 2.0% for both δ -tocotrienol (*Standard solution 1*) and γ -tocotrienol (*Standard solution 2*)

Chromatogram similarity: The chromatograms of *Standard solution 1* and *Standard solution 2* are similar to the reference chromatograms provided with the lot of [USP Annatto Seed Tocotrienols Extract RS](#) being used.

Analysis

Samples: Appropriate *Standard solution* and appropriate *Sample solution*

Calculate the content, in mg/g, of δ -tocotrienol in the portion of Annatto Seed Oil Tocotrienols taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W)$$

r_U = peak response of δ -tocotrienol from *Sample solution 1*

r_S = peak response of δ -tocotrienol from *Standard solution 1*

C_S = concentration of δ -tocotrienol in *Standard solution 1* (determined by applying the labeled amount of δ -tocotrienol content in the [USP Annatto Seed Tocotrienols Extract RS](#)) (mg/mL)

V = total volume used to prepare *Sample solution 1* (mL)

W = weight of Annatto Seed Oil Tocotrienols taken to prepare the *Sample stock solution* (g)

Calculate the content, in mg/g, of γ -tocotrienol in the portion of Annatto Seed Oil Tocotrienols taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W)$$

r_U = peak response of γ -tocotrienol from *Sample solution 2*

r_S = peak response of γ -tocotrienol from *Standard solution 2*

C_S = concentration of γ -tocotrienol in *Standard solution 2* (determined by applying the labeled amount of γ -tocotrienol content in the [USP Annatto Seed Tocotrienols Extract RS](#)) (mg/mL)

V = total volume used to prepare *Sample solution 2* (mL)

W = weight of Annatto Seed Oil Tocotrienols taken to prepare the *Sample stock solution* (g)

Calculate the percentage of total tocotrienols in the portion of Annatto Seed Oil Tocotrienols taken:

$$\text{Result} = [(D + G)/1000] \times 100$$

D = content of δ -tocotrienol (mg/g)

G = content of γ -tocotrienol (mg/g)

Calculate the percentage of δ -tocotrienol in the total tocotrienols:

$$\text{Result} = D/(D + G) \times 100$$

D = content of δ -tocotrienol in the portion of Annatto Seed Oil Tocotrienols (mg/g)

G = content of γ -tocotrienol in the portion of Annatto Seed Oil Tocotrienols (mg/g)

Calculate the percentage of γ -tocotrienol in the total tocotrienols:

$$\text{Result} = G/(D + G) \times 100$$

G = content of γ -tocotrienol in the portion of Annatto Seed Oil Tocotrienols (mg/g)

D = content of δ -tocotrienol in the portion of Annatto Seed Oil Tocotrienols (mg/g)

Acceptance criteria

Total tocotrienols: NLT 70.0% on the anhydrous basis

δ -Tocotrienol in total tocotrienols: 84%–92%

γ -Tocotrienol in total tocotrienols: 8%–16%

SPECIFIC TESTS

• **FATS AND FIXED OILS** (401), *Procedures, Peroxide Value*: NMT 5.0

• **WATER DETERMINATION** (921), *Method I, Method Ia*: NMT 1.0%

• LIMIT OF α -TOCOPHEROL

Mobile phase: 1,4-Dioxane and hexane (5:95)

Standard stock solution: 100 μ g/mL of [USP Alpha Tocopherol RS](#) in hexane

Standard solution: 0.6 μ g/mL of [USP Alpha Tocopherol RS](#) in *Mobile phase* from *Standard stock solution*

Sample solution: *Sample solution 2* from the test for [Content of \$\delta\$ - and \$\gamma\$ -Tocotrienols](#)

Chromatographic system: Proceed as directed in the test for [Content of \$\delta\$ - and \$\gamma\$ -Tocotrienols](#)

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for α -tocopherol

Relative standard deviation: NMT 2.0% for α -tocopherol

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatogram of the *Standard solution*, identify the peak corresponding to α -tocopherol in the *Sample solution*.

Calculate the percentage of α -tocopherol in the portion of Annatto Seed Oil Tocotrienols taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak response of α -tocopherol from the *Sample solution*