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^Ajowan Fruit Powder

DEFINITION

Ajowan Fruit Powder consists of the dried fruit of *Trachyspermum ammi* (L.) Sprague (Fam. Apiaceae), harvested in spring, reduced to powder or very fine powder. The dried fruit contains NLT 3.0% of volatile oils. It contains NLT 1.5% of thymol (C₁₀H₁₄O) and NLT 0.1% of 4-cymene (C₁₀H₁₄) on the anhydrous basis.

IDENTIFICATION

• **A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).**

- Standard solution A:** 1 mg/mL of [USP Thymol RS](#) in [methanol](#). Sonicate to dissolve.
- Standard solution B:** 20 mg/mL of [USP Trachyspermum ammi Fruit Oil RS](#) in [methanol](#)
- Sample solution:** Transfer 500 mg of Ajowan Fruit Powder into a 10-mL centrifuge tube. Add 5 mL of [methanol](#) and sonicate for 10 min. Centrifuge or filter and use the supernatant or the filtrate.

Chromatographic system

- (See standard parameters as defined in [HPTLC for Articles of Botanical Origin \(203\), Table 1.](#))
- Application volume:** 5 µL, as 8-mm bands
- Developing solvent system:** [Toluene](#) and [ethyl acetate](#) (95:5)
- Derivatization reagent:** Prepare an anisaldehyde reagent as follows. Slowly and carefully mix 170.0 mL of ice-cooled methanol with 20.0 mL of glacial acetic acid and 10.0 mL of sulfuric acid. Allow the mixture to cool to room temperature, and then add 1.0 mL of anisaldehyde.

Analysis

- Samples:** *Standard solution A, Standard solution B, and Sample solution*
- Apply the *Samples* as bands and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with the *Derivatization reagent* and dry in air for 5 min. Heat at 100° for 3 min. Examine under white light.
- System suitability:** *Standard solution B* exhibits an intense orange band due to thymol in the middle section corresponding in *R_F* and color to *Standard solution A*.
- Acceptance criteria:** The *Sample solution* exhibits an orange band due to thymol in the middle section corresponding in *R_F* and color to *Standard solution A*. One intense purple band appears above the thymol band and one pale purple band appears above that purple band. Four to five additional pale purple bands appear below the thymol band.

• **B. HPLC**

- Analysis:** Proceed as directed in the test for *Content of Thymol and 4-Cymene*.
- Acceptance criteria:** The *Sample solution* exhibits peaks at retention times corresponding to thymol and 4-cymene in *Standard solution A* and *Standard solution B*, with 4-cymene at about one-fifth the height of thymol.

COMPOSITION

• **CONTENT OF THYMOL AND 4-CYMENE**

- Solution A:** 0.2% [phosphoric acid](#) in [water](#)
- Solution B:** [Acetonitrile](#)
- Diluent:** See [Table 1](#).

Table 1

Time	Solution A (%)	Solution B (%)
0	75	25
10	45	55
17	20	
23	20	

Standard solution A: 0.05 mg/mL of USP Thymol RS and 0.03 mg/mL of USP 4-Cymene RS in methanol	25
Standard solution B: 1 mg/mL of USP Trachyspermum ammi Fruit Oil RS in methanol	
Sample solution: Transfer 500 mg of Ajowan Fruit Powder into a 100-mL flask and add 70 mL of methanol . Heat under reflux at 80° for 30 min. Transfer this solution to a 100-mL volumetric flask. Repeat the extraction process using 20 mL of methanol . Add methanol to volume. Pass through a filter of 0.45-µm pore size, discarding the first 2 mL of filtrate before injection.	

Chromatographic system(See [Chromatography \(621\), System Suitability](#).)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 10-cm; 2.7-µm packing [L1](#)**Column temperature:** 35°**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Samples:** *Standard solution A* and *Standard solution B***Suitability requirements****Resolution:** NLT 2.0 between the thymol peak and the previous peak, *Standard solution B***Tailing factor:** NMT 2.0 for the thymol and 4-cymene peaks, *Standard solution A***Relative standard deviation:** NMT 2.0% for the thymol and 4-cymene peaks in repeated injections, *Standard solution A***Chromatogram similarity:** The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Trachyspermum ammi Fruit Oil RS](#) being used.**Analysis****Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B* and the reference chromatogram provided with the lot of [USP Trachyspermum ammi Fruit Oil RS](#) being used, identify the retention times of the peaks corresponding to thymol and 4-cymene in the *Sample solution*.

Separately calculate the percentages of thymol and 4-cymene in the portion of Ajowan Fruit Powder taken:

$$\text{Result} = (r_u/r_s) \times C_s \times (V/W) \times 100$$

r_u = peak area of thymol or 4-cymene from the *Sample solution*

r_s = peak area of thymol or 4-cymene from *Standard solution A*

C_s = concentration of [USP Thymol RS](#) or [USP 4-Cymene RS](#) in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Ajowan Fruit Powder taken to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 1.5% of thymol and NLT 0.1% of 4-cymene on the anhydrous basis**CONTAMINANTS**

- **ARTICLES OF BOTANICAL ORIGIN (561)**, *Limits of Elemental Impurities*: Meets the requirements
- **ARTICLES OF BOTANICAL ORIGIN (561)**, *Pesticide Residue Analysis*: Meets the requirements
- **MICROBIAL ENUMERATION TESTS (2021)**: The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS (2022)**, *Test Procedures, Test for Absence of Salmonella Species* and *Test for Absence of Escherichia coli*: Meets the requirements

SPECIFIC TESTS• **BOTANICAL CHARACTERISTICS****Macroscopic:** Pale brown to grayish-brown powder

Microscopic: Coarse, oily, and sticky. Epicarpic cells with papillae-like projections as abundant elements and mesocarpic cells and oil globules. Cotyledon cells are also abundant and most of them contain oil globules. Epidermal cells are observed frequently and the spiral vessel elements are observed rarely. Oil globules are abundantly observed in almost all the fields.

- **ARTICLES OF BOTANICAL ORIGIN (561)**, *Methods of Analysis, Volatile Oil Determination*

Sample: 20 g of Ajowan Fruit Powder**Acceptance criteria:** NLT 3.0%

- **ARTICLES OF BOTANICAL ORIGIN (561)**, *Methods of Analysis, Alcohol-Soluble Extractives, Method 1*: NLT 7.0%
- **ARTICLES OF BOTANICAL ORIGIN (561)**, *Methods of Analysis, Water-Soluble Extractives*: NLT 14.0%
- **WATER DETERMINATION (921)**: NMT 7.0%