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Black Cumin Seed Thymoquinone Oil

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DEFINITION

Black Cumin Seed Thymoquinone Oil is the oil obtained from the seeds of *Nigella sativa* L. (Fam. Ranunculaceae) by cold press processing. It contains NLT 3% of thymoquinone, NLT 1% of 4-cymene, and NMT 0.1% of ▲carvacrol▲ (ERR 1-Sep-2023). It may contain added substances as stabilizers.

IDENTIFICATION

• **A. [IDENTIFICATION OF FIXED OILS BY THIN-LAYER CHROMATOGRAPHY \(202\)](#):** The R_f values of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.

• **B. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#).**

Standard solution A: Prepare individual solutions: 1 mg/mL of [USP Thymoquinone RS](#) in [methanol](#) and 2 mg/mL of [USP Carvacrol RS](#) in [toluene](#). Sonicate to dissolve. Apply each of these solutions onto the plate by overspotting.

Standard solution B: 20 mg/mL of [USP Black Cumin Seed Oil RS](#) in [acetone](#). Sonicate to dissolve.

Sample solution: 20 mg/mL of Black Cumin Seed Thymoquinone Oil in [acetone](#). Sonicate to dissolve.

Chromatographic system

Absorbent: [Silica gel, precoated plates, with fluorescence indicator F₂₅₄](#)

Application volume: 2 μ L, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: About 25°

Developing solvent system: [Toluene](#), [cyclohexane](#), and [acetone](#) (8: 2: 0.5)

Developing distance: 6 cm from the application line

Derivatization reagent: 170 mL of ice-cooled [methanol](#) mixed with 20 mL of [glacial acetic acid](#), 10 mL of [sulfuric acid](#), and 1 mL of [p-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. Examine the plate at 254 nm. Treat the plate with *Derivatization reagent*, dry the plate at 100° for 3 min, and examine under white light and under long wavelength UV light.

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

UV light at 254 nm: Prior to derivatization and under UV light at 254 nm, *Standard solution A* exhibits a quenching band in the upper-third section due to thymoquinone. *Standard solution B* exhibits a quenching band corresponding in R_f to thymoquinone in *Standard solution A*.

White light: After derivatization and under white light, *Standard solution A* exhibits an orange band and a faint brown band corresponding in increasing R_f to carvacrol and thymoquinone, respectively. *Standard solution B* shows a faint brown band corresponding in R_f to thymoquinone in *Standard solution A*. *Standard solution B* also exhibits an intense purple band in the upper-third section, one to two light purple bands below thymoquinone in *Standard solution A*, and three purple bands in the lower-third section below carvacrol in *Standard solution A*.

Long wavelength UV light: After derivatization and under long wavelength UV light, *Standard solution A* exhibits a black band and a blue band corresponding in increasing R_f to carvacrol and thymoquinone, respectively. *Standard solution B* exhibits a blue band corresponding in R_f to thymoquinone in *Standard solution A*. *Standard solution B* also exhibits an intense orange band in the upper-third

section, a light orange band and a light purple band below thymoquinone in *Standard solution A*, and three dusty pink bands in the lower-third section below carvacrol in *Standard solution A*.

Acceptance criteria

UV light at 254 nm: Prior to derivatization and under UV light at 254 nm, the *Sample solution* exhibits a quenching band in the upper-third section due to thymoquinone in *Standard solution A*, which is also observed in *Standard solution B*.

White light: After derivatization and under white light, the *Sample solution* shows a faint brown band corresponding in R_f and color to thymoquinone in *Standard solution A*. The *Sample solution* also exhibits one to two light purple bands below thymoquinone, three purple bands in the lower-third section, and an intense purple band in the upper-third section, corresponding to the same bands in *Standard solution B*.

Long wavelength UV light: After derivatization and under long wavelength UV light, the *Sample solution* exhibits a blue band corresponding in R_f to thymoquinone in *Standard solution A*. The *Sample solution* also exhibits a light orange band and a light purple band below thymoquinone, three pink bands in the lower-third section, and an intense orange band in the upper-third section, corresponding to the same bands in *Standard solution B*.

• C. GC PROFILE OF MONOTERPENES

Analysis: Proceed as described in the test for *Content of Monoterpenes*.

Acceptance criteria: The *Sample solution* exhibits peaks corresponding to the retention times of 4-cymene, thymoquinone, and carvacrol in *Standard solution A* and *Standard solution B*.

- **D.** It meets the requirements in the specific test for *Fats and Fixed Oils, Fatty Acid Composition*.

COMPOSITION

• CONTENT OF MONOTERPENES

Standard solution A: 50 µg/mL each of [USP Thymoquinone RS](#) and [USP 4-Cymene RS](#) and 5 µg/mL of [USP Carvacrol RS](#) in [acetone](#)

Standard solution B: 2 mg/mL of [USP Black Cumin Seed Oil RS](#) in [acetone](#)

Sample solution: 2 mg/mL of Black Cumin Seed Thymoquinone Oil in [acetone](#)

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused silica capillary; coated with 0.25-µm film of phase [G27](#)

Temperatures

Injection port: 250°

Detector: 270°

Column: See [Table 1](#).

Table 1

Initial Temperature (°)	Hold Time (min)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
105	5	25	260	3.8

Carrier gas: Helium

Flow rate: 2 mL/min

Injection volume: 1 µL

Injection type: Split, split ratio 1:10

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 5 between thymoquinone and carvacrol peaks, *Standard solution A*

Tailing factor: NMT 1.5 for each analyte, *Standard solution A*

Relative standard deviation: NMT 2.0% for each analyte, *Standard solution A*

Chromatogram similarity: The chromatogram of the *Sample solution* is similar to the reference chromatogram provided with the lot of [USP Black Cumin Seed Oil RS](#) being used.

Analysis

Samples: *Standard solution A* and *Sample solution*