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Anise Oil

CAS RN®: 8007-70-3.

DEFINITION

Anise Oil is the volatile oil distilled with steam from the dried, ripe fruit of *Pimpinella ansium* L. (Family Apiaceae). It contains NLT 87% and NMT 94% of *trans*-anethole. [Note—If solid material has separated, carefully warm the Anise Oil until it is completely liquefied, and mix before using.]

IDENTIFICATION

Change to read:

• **A. Chromatographic Identity**

Peak identification and sensitivity solution: 60 mg/mL of USP Anethole RS, 3 mg/mL of USP Pseudoisoeugenyl 2-methylbutyrate RS, and 0.1 mg/mL each of USP Foeniculin RS and USP Safrole RS in *n*-hexane

Standard: USP Anise Oil RS

Sample: Anise Oil

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m; coated with 0.25-µm film of phase G16

Temperatures

Injection port: 200°

Detector: 220°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	—	60	5
60	2	210	15

Carrier gas: Helium

Flow rate: 1.0 mL/min

Injection volume: 0.4 µL

Injection type: Split, split ratio 100:1

System suitability

Sample: *Peak identification and sensitivity solution*

[Note—The relative retention times for the *trans*-anethole, safrole, foeniculin, and pseudoisoeugenyl 2-methylbutyrate peaks are 1.0, 1.04, 1.5, and 1.7, respectively.]

Suitability requirements

Signal-to-noise ratio: NLT 10 for the foeniculin and safrole peaks

Analysis

Samples: *Peak identification and sensitivity solution*, *Standard*, and *Sample*

Identify the safrole, foeniculin, and pseudoisoeugenyl 2-methylbutyrate peaks in the chromatogram of the *Sample* based on those in the chromatogram of the *Peak identification and sensitivity solution*.

Calculate the percentage of safrole, foeniculin, and pseudoisoeugenyl 2-methylbutyrate in the portion of Anise Oil taken:

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

r_U = peak area of safrole, foeniculin, or pseudoisoeugenyl 2-methylbutyrate from the *Sample*

r_T = sum of all the peak areas from the *Sample*, excluding the solvent peak

Acceptance criteria

Chromatographic similarity: Examine the chromatograms of the *Sample* and the *Standard*. The chromatogram of the *Sample* is similar to that of the *Standard*. [NOTE—The chromatogram of the *Standard* is similar to the reference chromatogram provided with the lot of ▲USP Anise Oil RS▲ (ERR 1-Nov-2020) being used.]

Safrole: NMT 0.01%

Foeniculin: NMT 0.01% [NOTE—In case of a failing result, confirm the presence of foeniculin by GC-MS using the *Chromatographic system* parameters specified in this test. An interfering peak eluting at or near the retention time of foeniculin may be myristicin. Mass to charge values for molecular and major fragment ions of foeniculin and myristicin obtained with Electron Ionization (EI) and Chemical Ionization (CI) are presented in *Table 2*. When the absence of foeniculin is confirmed then the *Sample* meets the acceptance criteria.]

Table 2

Compound	EI, $M^{\cdot +}$		CI, $[M+H]^+$	
	Molecular Ion (m/z)	Major Fragment Ion (m/z)	Molecular Ion (m/z)	Major Fragment Ion (m/z)
Foeniculin	202.14	134.07	203.14	135.08
Myristicin	192.08	91.05	193.08	165.09

Pseudoisoeugenyl 2-methylbutyrate: NLT 0.30%

• **B. REFRACTIVE INDEX:** Meets the requirements for *Refractive Index* in *Specific Tests*

ASSAY

• **CONTENT OF *trans*-ANETHOLE**

Peak identification and sensitivity solution, Sample, and **Chromatographic system:** Proceed as directed in *Identification A*.

Analysis

Samples: *Peak identification and sensitivity solution* and *Sample*

Identify the *trans*-anethole peak in the chromatogram of the *Sample* based on that in the chromatogram of the *Peak identification and sensitivity solution*.

Calculate the percentage of *trans*-anethole in the portion of Anise Oil taken:

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

r_U = peak area of *trans*-anethole from the *Sample*

r_T = sum of all the peak areas from the *Sample*, excluding the solvent peak

Acceptance criteria: 87%–94%

IMPURITIES

• **LIMIT OF PHENOLS**

Sample solution: Recently distilled Anise Oil in 90% alcohol (1 in 3)

Acceptance criteria: The *Sample solution* is neutral to moistened neutral litmus paper, and no blue or brownish color develops upon the addition of 1 drop of ferric chloride TS to 5 mL of the *Sample solution*.

SPECIFIC TESTS

• **SOLUBILITY IN 90% ALCOHOL:** 1 volume dissolves in 3 volumes of 90% alcohol.

• **REFRACTIVE INDEX ⟨831⟩:** 1.553–1.560 at 20°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-filled, tight containers, and protect from light. Avoid exposure to excessive heat.

• **LABELING:** The label states the Latin binomial name and, following the official name, the part of the plant source from which the article was derived. The label also states that if solid material has separated, carefully warm the oil until it is completely liquefied, and mix before using.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Anethole RS
USP Anise Oil RS
USP Foeniculin RS

USP Pseudoisoeugenyl 2-methylbutyrate RS
USP Safrole RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ANISE OIL	Documentary Standards Support	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

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