⟨202⟩ IDENTIFICATION OF FIXED OILS BY THIN-LAYER CHROMATOGRAPHY

INTRODUCTION

The following procedure for the USP *Identification* test is used to identify fixed oils using high-performance thin-layer chromatography (HPTLC), with a suitable octadecylsilyl silica gel as the coating substance.

IDENTIFICATION

• **METHOD I**

Mobile phase 1: Ethyl ether

Mobile phase 2: Methylene chloride, glacial acetic acid, and acetone (20:40:50)

System suitability solution 1: Dissolve about 20 mg (1 drop) of USP Corn Oil RS in 3 mL of methylene chloride.

System suitability solution 2: Dissolve about 20 mg (1 drop) of USP Olive Oil RS in 3 mL of methylene chloride.

Standard solution: Dissolve about 20 mg (1 drop) of the appropriate USP Reference Standard in 3 mL of methylene chloride.

Sample solution: Dissolve about 20 mg (1 drop) of a fixed oil sample in 3 mL of methylene chloride.

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)

Mode: HPTLC

Plate: 20 cm × 10 cm, silica gel 60 RP-18, 0.15–0.2 mm layer, 4–8 µm particle size[1]

Application volume: 1 µL, manually spot

Spray reagent: 100 mg/mL of phosphomolybdic acid in 96% alcohol

System suitability

Samples: *System suitability solution 1* and *System suitability solution 2*

Suitability requirements

Resolution: The four principal spots from corn oil are clearly identified and separated, and the two principal spots from olive oil are clearly identified and separated.

[NOTE—Retardation factors (R_F) are provided for informational purposes only to aid in spot identification. The R_F values for the four principal spots for USP Corn Oil RS are 0.39, 0.45, 0.51, and 0.56, and the R_F values for the two principal spots for USP Olive Oil RS are 0.39 and 0.45.]

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* in separate bands to the previously marked starting point on an HPTLC plate, and develop the plate in the following order:

1. Ensure that the spots are at least 3 mm above the surface of the mobile phase. Develop two times over a path of 0.5 cm using *Mobile phase 1*. Remove the plate from the chamber after each run, and allow the plate to dry in air.
2. Develop two times over a path of 8 cm using *Mobile phase 2*. Allow the plate to dry for about 5 min after each development and before spraying with a spray reagent.

Spray the plate with the *Spray reagent*.

Heat the plate at 120° for about 1 min, and examine in daylight.

Acceptance criteria: The R_F values of the principal spots of the *Sample solution* correspond to those of the *Standard solution*.

• **METHOD II**

Mobile phase: Methylene chloride, glacial acetic acid, and acetone (20:40:50)

System suitability solution 1: Dissolve 25 µL of USP Corn Oil RS in 3 mL of methylene chloride.

System suitability solution 2: Dissolve 25 µL of USP Olive Oil RS in 3 mL of methylene chloride.

Standard solution: Dissolve 25 µL of the appropriate USP Reference Standard in 3 mL of methylene chloride.

Sample solution: Dissolve 25 µL of a fixed oil sample in 3 mL of methylene chloride.

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)

Mode: HPTLC

Plate: 20 cm × 10 cm, silica gel 60 RP-18 (or RP-18 F_{254}), 0.15–0.2 mm layer, 4–8 µm particle size

Conditioning of plate: Condition the plate to a relative humidity of about 33%.

Application volume: 2 µL as bands of 8 mm. A suitable automated apparatus is used.

Spray reagent: 25 mg/mL of phosphomolybdic acid in 96% alcohol

System suitability

Samples: *System suitability solution 1* and *System suitability solution 2*

Suitability requirements

Resolution: The four principal bands from corn oil are clearly identified and separated, and the two principal bands from olive oil are clearly identified and separated.

[NOTE—Retardation factors (R_F) are provided for informational purposes only to aid in band identification. The R_F values for the four principal bands for USP Corn Oil RS are 0.20, 0.26, 0.30, and 0.34, and the R_F values for the two principal bands for USP Olive Oil RS are 0.20 and 0.26.]

Analysis

Samples: *Standard solution* and *Sample solution*

Predevelop the plate with methylene chloride to the upper edge. Dry the plate at 120° for 10 min.

[1] HPTLC Silica Gel 60 RP-18 plate from Merck EMD, or equivalent.

Apply the *Samples* in separate bands to the previously marked starting point on an HPTLC plate.
Ensure that the bands are at least 3 mm above the surface of the mobile phase.
Develop over a path of 7 cm using *Mobile phase*. Allow the plate to dry in air.
Treat the plate with the *Spray reagent*.
Heat the plate at 120° for 3 min, and examine in daylight.

Acceptance criteria: The R_F values of the principal bands of the *Sample solution* correspond to those of the *Standard solution*.

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Almond Oil RS
USP Borage Seed Oil RS
USP Canola Oil RS
USP Corn Oil RS
USP Cottonseed Oil RS
USP Evening Primrose Oil RS
USP Flax Seed Oil RS
USP Olive Oil RS
USP Palm Oil RS
USP Peanut Oil RS
USP Safflower Oil RS
USP Sesame Oil RS
USP Soybean Oil RS
USP Sunflower Oil RS