APPLICATION NOTES



HPTLC Identification of Fatty Oils (Fixed Oils)

F-39









Scope:

This Application Note illustrates the United States Pharmacopeia's (USP) chapter <202> general Identification Fixed Oils of by Thin-Laver Chromatography which includes an HPTLC method for the identification of 14 different fatty oils. The method was optimized for reliable and reproducible visual identification and is based on pre-washed RP-18 HPTLC plates, development in an automatic developing chamber (ADC2) and detection by immersion in phosphomolybdic acid reagent.

Sample:

 $25\,\mu L$ of fatty oil are dissolved in $3\,m L$ of dichloromethane (methylene chloride). This is the sample solution.

System suitability test (SST):

Solution 1: Dissolve 25 μL of USP Corn Oil RS in 3 mL of dichloromethane.

Solution 2: Dissolve 25 μL of USP Olive Oil RS in 3 mL of dichloromethane.

Standards:

Dissolve 25 μ L of the appropriate USP Fixed Oil RS in 3 mL of dichloromethane.

Derivatization reagent:

Chromatographic conditions:

25 mg/mL of phosphomolybdic acid in 96% ethanol

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Stationary phase:

HPTLC plates RP-18 (Merck), 20x10 cm; Pre-develop the plate with dichloromethane to the upper edge. Dry the plate at 120°C for 10 minutes.

Mobile Phase:

Sample application:

Dichloromethane, glacial acetic acid, acetone (20:40:50)

 $2~\mu L$ of sample solution, SST solution and standard solution as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.

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Development: 20x10 cm Twin Trough Chamber (ADC2), saturated for

20 min (filter paper), developing distance 80 mm from lower edge of plate. The plate is dried with cold air for 5 minutes. The plate is conditioned to a relative humidity

of about 33%.

Derivatization: The plate is immersed into the derivatization reagent for

one second and heated for 3 minutes at 120°C.

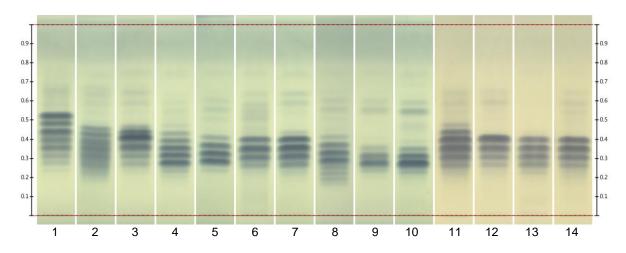
Detection: Examination in white light.

Acceptance criteria:

The Rf values of the principal bands of the sample solution correspond to those of the standard solution.

Results:

Compare to the chromatogram below:



- 1: USP Flax seed oil RS
- 2: USP Borage seed oil RS
- 3: USP Evening primrose oil RS
- 4: USP Canola oil RS
- 5: USP Almond oil RS
- 6: USP Cottonseed oil RS 7: USP Corn oil RS
- 0. LICE Departs all D
- 8: USP Peanut oil RS 9: USP Palm oil RS
- 10: USP Olive oil RS
- 11: USP Soybean oil RS
- 12: USP Safflower oil RS
- 13: USP Sesame oil RS
- 14: USP Sunflower oil RS

References:

 Modified from <202> Identification of Fixed Oils by Thin-Layer Chromatography, Pharmacopeial Forum 39(3)