


TESTING PROCEDURE			
No. TM 01	Thin Layer Chromatography of Foeniculi Vulgaris Fructus	Revision No.: 0	
Date Issued 27 Nov 2015	Department Quality Control	Effective Date <i>1 January 2016</i>	
Prepared by : QC Section Head		Approved by : Quality Control Manager	Page: 1 of 2

1. Apparatus

- Chromatography chamber
Chamber can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate-support rack that supports the plates, back to back, with the lead of the chamber in place.
- Template
To guide in placing the test spots at definite intervals, to mark distances as needed, and to aid in labelling the plates.
- Micropipets
Deliver quantities volume of fluid.
- Reagent sprayer
Produce fine spray and will not itself be attacked by the reagent.
- UV light source
Suitable for observations under 254 upto 366 nm wavelength UV light.

2. Reagent

- Toluene R
- Ethyl acetate R
- Ethanol 95% R
- Standard : *Trans*-anetol


3. Procedure

TLC Condition :

- | | |
|--------------------|--|
| Mobile Phase | : Toluene R– ethyl acetate R (90:10) |
| Stationary Phase | : Plate with silica gel 60 F ₂₅₄ |
| Test Solution | : 10% in ethanol 95% R |
| Reference Solution | : 1% <i>Trans</i> – anetolin ethanol 95% R |
| Volume of Spot | : Apply separately 20 µL Test Solution and 2 µL Standard Solution on the plate |
| Detection | : UV ₃₆₆ |

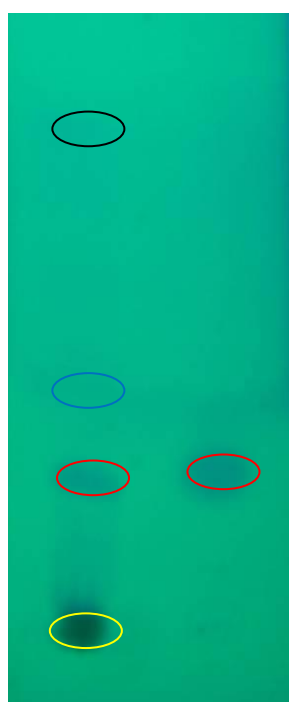
Chamber saturation :

Place filter paper into chromatography chamber. The height of filter paper should be adjusted with the size of chromatography chamber. Pour into the chromatography chamber a quantity of the mobile phase to a depth of 0.5 to 1 cm from the bottom of the chamber. Seal the lid at the top of chamber and let the filter-paper become completely impregnated. Make sure that the tip of the filter paper is always immersed in the mobile phase. Unless otherwise specified at the monograph, TLC is performed in a saturated chamber.

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TLC Procedure :

Apply the prescribed test solution and standard solution, add approximately 1.5 cm and 2 cm from the lower edge the plate, and allow to dry. Use a template to guide placing the test spots and mark distances, label as indicated. Arrange the plate on the supporting rack, test spots toward the bottom, and place the rack in the chromatography chamber. Ensure the mobile phase reach the lower edge of the adsorbant, but the spots or bands are above the surface of the mobile phase. Close the chamber and maintain the system until the mobile phase has moved over the prescribed distance. Remove and air-dry the plate, and visualize the chromatogram under visible light with long-wavelength UV light (366 nm). Measure and record the distance of each spot or zone from the point of origin, and indicate for each spot or zone the wavelength under which it was observed. Determine the R_f of standard solution and sample. Observe and compare the test chromatogram with the standard chromatogram.



Sample Standard

Sample	Standard
R _f = 0.15	R _s = 0.25
R _f = 0.25	
R _f = 0.40	
R _f = 0.80	