Appendix IV(A): Chromatography - Thin-Layer Chromatography (TLC)

TLC is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support (plate) of glass, plastic or aluminum film. Standard and test solutions are applied separately on the plate and the components are separated by the developing solvent systems. For the identification of CMM, separated spots obtained from the test solution are compared with the corresponding spots obtained from the chemical reference substance(s) in the chromatogram.

(1) **Apparatus and materials –**

(a) **TLC plates** – The most commonly used coated plates are silica gel G, silica gel F_{254}, HPTLC silica gel F_{254}, silica gel H and silica gel HF_{254}. Diatomaceous earth, diatomaceous earth G, aluminum oxide, aluminum oxide G, microcrystalline cellulose and microcrystalline cellulose F_{254} etc., can be used as well. Coated plates with the size of 10×5 cm; 10×10 cm; 10×15 cm; 20×10 cm or 20×20 cm are commonly used.

(b) **Application devices** – Micropipettes, micro-syringes, calibrated capillaries or other suitable application devices can be used for the proper application of solutions to the plates.

(c) **Developing chamber** – A tank of size suitable for the plates, with a tightly fitting lid and with a flat bottom or twin trough is usually used.

(d) **Spray reagents** – Spray reagent for the detection of spots is specified in the individual monograph.

(e) **Ultraviolet (UV) light source** – An emitting light source in the UV range is used for the examination of spots in the chromatogram.

(2) **Procedure** –

(a) **Saturation of the developing chamber** – Unless otherwise specified, carry out the chromatography in a saturated chamber. To achieve saturation, pour sufficient amount of the developing solvents into the developing chamber, replace the lid and allow it to stand for 15-30 min at room temperature. If necessary, line the inner walls of the developing chamber with filter paper strips, the lower edges of the filter papers should be immersed in the developing solvents.

(b) **Applying the standard and test solutions** – Apply separately the prescribed volumes of the standard and test solutions in small portions to obtain spots (less than 3 mm in diameter) or bands on a line parallel to, and 15 mm from, the lower edge of the plate. Pay attention not to apply spots or bands less than 15 mm from the sides of the plate and no disturbance of each other should occur.

(c) **Developing a chromatogram** – Place the plate in the chromatographic tank after the solvent has evaporated from the applied solutions, ensuring that the sample line are 5 mm above the surface of the developing solvents. Then cover the chamber tightly with a lid. Remove the plate from the chamber when the developing solvents have moved over the distance as prescribed in the individual monograph. Dry the plate and visualize the chromatogram under visible light and/or UV light as specified in the individual monograph.

(d) **Interpretation of the chromatogram** – Compare the principal spots or bands observed from the test solution with the corresponding spots or bands observed from the standard solutions. For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f value, similar to those of the chemical reference substances when observed under visible light and/or UV light as specified in the individual monograph.

The R_f value is defined as the ratio of the distance from the point of application to the centre of the spot or band to the distance travelled by the solvent front from the point of application:

$$R_f = \frac{\text{Distance from the point of application to the centre of the spot or band}}{\text{Distance travelled by the solvent front from the point of application}}$$