

without removing the cover by carefully tipping the chamber to transfer mobile phase to the side with the plate. In a third mode, a different solvent or humidity-controlling sulfuric acid–water mixture is put on one side and the plate and mobile phase on the other to provide gas-phase conditioning during development. Desaga sells a thermostatted chamber for control of temperature during development (118) (see Sec. VII.G).

Sandwich chambers (S-chambers), which have a second glass plate placed about 1 mm from the surface of the layer and therefore a very small gas space, can be used unsaturated, or saturated through the presence of a mobile-phase-soaked counter layer or filter paper sheet or pad. S-chambers today are mostly horizontal, and the Camag Linear Development Chamber and Desaga H-Separation Chamber are examples. The Camag chamber allows development of 70 samples simultaneously from both ends toward the center on a 20×10 cm HPTLC plate, or one-half that number of samples can be developed over the full length of the plate from one end only. Ascending and horizontal S-chambers have been described (144).

The Camag HPTLC Vario System is a horizontal chamber that has a wide variety of operational modes and applications. The plate is placed layer down over a tray with various compartments, which can hold different mobile phases, humidity controlling liquids, and volatile acids and bases whose vapors will impregnate and condition or preload the layer. Developing solvent is in a separate tray and is transferred to the layer by a wick. The Vario chamber can be used to test six mobile phases side by side on one plate for solvent optimization, to determine if layer pre-equilibration (preloading) is advantageous, to ascertain if S- or N-chamber configuration is best, and to test different humidity conditions.

Two new techniques involving horizontal development have been described. In “halfway development,” a new horizontal sandwich chamber is used to apply mobile phase to any part of a plate in order to redevelop a separated zone. This chamber can also be used for relay development of an overlength plate, programmed multiple development, gradient development, band application, concentration, and micropreparative separation (144a). Flow TLC (FTLC) involves sample injection into mobile phase flowing over a horizontal layer with continuous optical detection at a fixed layer position. Mobile phase is evaporated from the end of the plate to maintain constant mobile-phase velocity depending on capillary effects (144b). Practical applications of these new techniques have not yet been demonstrated.

Ascending development of TLC sheets has been carried out in plastic bags for quality screening of pharmaceuticals under field conditions (145).

Displacement TLC uses three different mobile phases (carrier, displacer, and regenerant) and three main steps (loading the sample; development of the displacement train, collecting the fractions of the separated bands; and regeneration). The displacement system is generated in situ, when the mixture of carrier and displacer is separated as a result of the carrier running faster than the displacer–carrier mixture. The principles, techniques, and possibilities of displacement TLC have been described (146), but few practical applications have been reported.

B. Circular and Anticircular Development

Circular or radial development was first carried out in a Petri dish containing mobile phase and a wick that touches the layer, supported on top of the dish, at its central point. The Camag U-Chamber and Anticircular U-Chamber have been used for circular and anticircular development, respectively, but these instruments are no longer listed in the Camag catalog. Except for the very occasional publication of research employing circular development (e.g., Refs. 147, 148), the method is almost never used currently for analytical TLC. However, circular chromatograms are produced by use of the modern preparative methods, e.g., multilayer OPLC (ML-OPLC) and micropreparative U-RPC (149).

C. Multiple Development

Thin-layer chromatography with multiple development often allows separation of complex mixtures or closely related substances not resolvable with a single development. The plate is repeatedly developed in the same direction, with the mobile phase dried between runs. Each