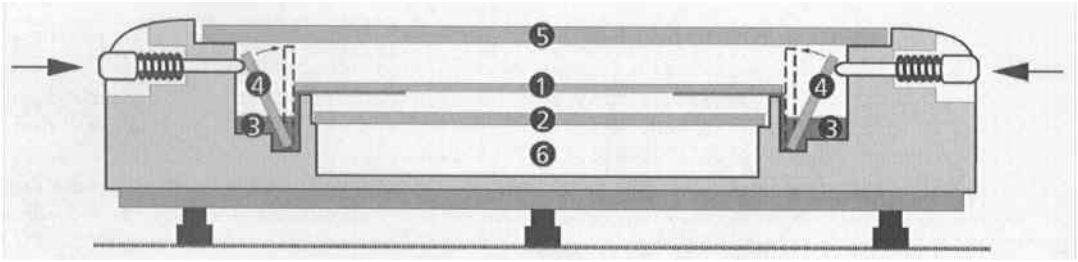

Figure 8 Schematic of the Horizontal Developing Chamber (HDC) (CAMAG, Muttenz, Switzerland). *1*, HPTLC plate (layer facing down); *2*, glass plate for sandwich configuration; *3*, reservoir for developing solvent; *4*, glass strip; *5*, cover plate; *6*, conditioning tray. The HPTLC plate is placed into the chamber with the layer facing down. The reservoir (*3*) is charged with developing solvent. The plate can be developed horizontally either from one side only or from opposite sides simultaneously, in this way doubling the number of samples per plate. Chromatography is started when the glass strip (*4*) is brought into a vertical position. In the unsaturated configuration, the conditioning tray (*6*) is empty; the glass plate (*2*) is removed. In the saturated configuration, the conditioning tray (*6*) contains developing solvent; the glass plate (*2*) is removed. For preconditioning, the conditioning tray (*6*) contains conditioning liquid; the glass plate (*2*) is removed. Development is started after preconditioning is completed. In the sandwich configuration, the conditioning tray (*6*) is empty; the glass plate (*2*) is in place.

developing solvent]. After a certain conditioning time, developing solvent is introduced into the front trough that contains the plate.

The ultimate versatility is achieved with the horizontal developing chamber of CAMAG (Fig. 8), which is designed for either 10 × 10 cm or 20 × 10 cm HPTLC plates. Not only are several configurations (saturated, unsaturated, preconditioned, sandwich) possible, but also development of samples from opposite sides of the plate. Applied as spots, up to 72 samples can be simultaneously chromatographed on a 20 × 10 cm plate. By using the center tray of the chamber for conditioning, the relative humidity during chromatographic separation can be controlled.

For method development and optimization of chromatographic parameters, the HPTLC-Vario chamber (CAMAG) is the ideal tool. Up to six different solvents or six different conditions can be used simultaneously on 10 × 10 cm HPTLC plates that have to be scored for this purpose with a special device. The optimized system can easily be transferred to a horizontal developing chamber.

C. Automated Multiple Development

Automated multiple development (AMD) a step-gradient technique derived by Burger (8), achieves the maximum resolution feasible within the limited separation distance available on an HPTLC plate. In terms of peak capacity, it compares with HPLC while retaining the inherent benefits of planar chromatography. Unlike a gradient in column chromatography, an AMD gradient starts with the solvent having the strongest elution power. In successive runs the solvent is varied toward decreasing elution power, and each run proceeds to a higher migration distance than the previous one. Typical distance increments are 3 mm or less for a 20–25-step gradient. Between developments, the solvent is completely removed from the chamber and the layer is dried under vacuum. Preconditioning through the gas phase prior to development is possible (9). "Universal gradient" is the term for an AMD gradient that starts with a very polar solvent and is varied via a solvent of medium polarity to a nonpolar solvent. Depending on solubility considerations, methanol or acetonitrile is typically used as the most polar component. The central or "base" solvent and, to a certain extent, the nonpolar solvent determine the selectivity of the separation. A solvent such as dichloromethane or *t*-butyl methyl ether is used as the base solvent in most AMD applications. Solvents for AMD must meet two requirements: They must be suitable for being dried off by vacuum, and they must be pure.