tance each time, and the last front migration distance is the longest, corresponding to the useful development length of the chromatoplate and the mobile phase.

3. Gradient MD (GMD) is a multiple development technique where the successive chromatographic development steps are performed with a change in solvent strength and selectivity (S_{T1}, $S_{V1} \rightarrow S_{T5}$, S_{V5}) for the same chromatographic length (D = const.) (see Fig. 6).
4. Bivariate MD (BMD) is the most complex multiple development technique. The development distance and mobile phase composition vary simultaneously (D_1, S_{T1}, $S_{V1} \rightarrow D_5$, S_{T5}, S_{V5}) during successive chromatographic runs (see Fig. 6).

The advantages of MD techniques are summarized in Ref. 86.

G. Selection of Other Operating Parameters

In TLC the solvent velocity is one parameter that, in principle, cannot be influenced by the chromatographer. Compared to TLC where the mobile phase migrates only by capillary action, the enhanced efficiency of FFPC techniques is due to the constant linear mobile phase velocity. FFPC techniques guarantee optimal H/u values. In OPLC the upper limit of velocity depends on the applied external pressure as well as on the viscosity. In RPC, the higher the rotational speed, the faster the migration of the mobile phase. The local mobile phase velocity can be influenced by the selection of the development mode.

In TLC the separation distance improves with the square root of the separation distance. However, the optimum depends on the quality of the plate (average particle size and size distribution of the stationary phase), the vapor space, the development mode, and the properties of the compounds to be separated.

In OPLC, because of the forced flow of eluent, the developing distance can be increased to about 18 cm by using a fine-particle sorbent layer by maintaining the high efficiency of separation.

A realistic possibility of increasing the efficiency and rapidity of planar chromatography is a novel aspect of multilayer OPLC; in long-distance OPLC (65,66) the efficiency of the separation is increased significantly. With this technique the end of the first chromatoplate has a slitlike perforation to permit the mobile phase to migrate to a second layer. A long separation distance can be achieved by adding one plate to another (87,88).

Sample application is one of the most important steps for a successful planar chromatographic separation; this has been summarized by Kaiser (89).

During method development, the applied development mode, forced-flow technique, and development distance always depend on the separation distance. This is summarized in Fig. 7 in the form of a flow chart.

IV. DETECTION AND QUANTIFICATION

A. Conventional Detection Modes

For the visualization of compounds, one can use physical, chemical, or biological detection methods. Physical detection methods are based on substance-specific properties. The most commonly employed methods are the absorption or emission of electromagnetic radiation, which is measured by detectors. The β-radiation of radioactively labeled compounds can also be detected directly on the plate. These nondestructive detection methods allow subsequent isolation of compounds and can also be followed by microchemical and/or biological detection methods (90). Because physical detection methods are frequently not sufficient to establish the identity of compounds, they must be complemented by specific chemical reactions (derivatization). These reactions may be carried out either before or after chromatography.

Prechromatographic derivatization can be done either during sample preparation or on the chromatoplate. It is generally used to introduce a chromophore, leading to the formation of strongly absorbing or fluorescent derivatives; to increase the selectivity of the separation; to enhance the sensitivity of detection; and to improve the linearity. It includes oxidation, reduction,