A 25-step gradient based on methanol, diethyl ether, and hexane was used to separate the six major human plantar stratum corneum lipids (71). Peak heights as well as peak areas were used for densitometric quantification of separated lipids.

AMD-HPTLC gradient development enabled the separation and quantification of forskolin and its 10 derivatives (72). These diterpenoids have interesting pharmacological properties.

Multistep gradient elution can also be carried out with modified overpressured TLC equipment (20,74), described in Chapter 7 of this Handbook. Vajda et al. (20) applied the method to the analysis of the components of total lipid extracts from various human blood samples. Pick (74) used it for the chromatographic separation of membrane gangliosides. The advantage of the procedure consists in the removal of less polar solutes in the first stages of the gradient and separation of the polar gangliosides in the last stages.

IV. OPTIMIZATION OF STEPWISE GRADIENT ELUTION

A. Graphical Method

Consider the elution of a given solute by a two-component mobile phase on a chromatographic plate during stepwise gradient elution (10,12). The length of the plate is assumed to be unity. The composition of the binary mobile phase is defined in terms of the concentration of the stronger solvent. It is assumed that the composition of the mobile phase changes gradually during elution but is constant in each step. The elution model is presented in Fig. 12 (12).

Assuming a constant mobile-phase flow rate, the straight line $0Y$ shows the migration of the mobile-phase front. The migration rate of compound A is lower than that of the mobile phase, and after one dead volume of eluent has passed through the bed, the R_f value of compound A is 0.2 (point A in Fig. 12). When the front of the mobile phase of 5% concentration reaches the end of the plate (R_f = 1.0), the concentration of the eluent is changed stepwise. The solvent front is observed by means of a marker (azulene or azobenzene) whose R_f value in the solvent system is close to unity. The line $0'Y'$ in Fig. 12 indicates the migration of the mobile phase of 10% concentration. Obviously, the front of 10% mobile phase will, after some time, overtake spot A, which traveled until then in the mobile phase of 5% concentration (section AA'). From point A' onward, the spot travels in the mobile phase of 10% concentration. It is assumed that the R_f value for compound A in the mobile phase of 10% concentration is 0.3. To find point B, a length $A'C$ corresponding to one dead volume V_m is marked, and a section equal to 0.3 R_f unit from point C is measured. Upon connecting points A', B, B', the migration of the spot A in the 10% mobile phase and the final R_f value are obtained.

If the R_f values obtained in several isocratic elution steps are known, the program for gradient elution can be constructed (11,12). Results of stepwise gradient elution of DABS-amino acids are

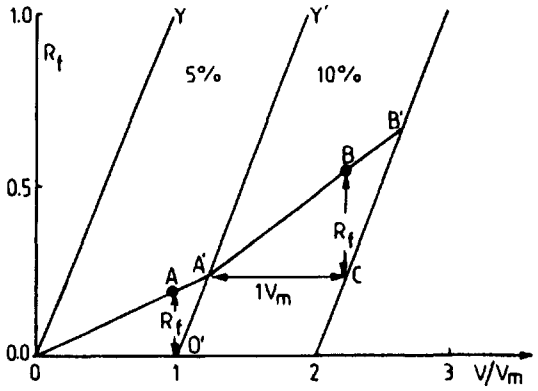

Figure 12 Graphical representation of the movement of sample A during stepwise gradient elution. (Reprinted from Ref. 12 with permission.)