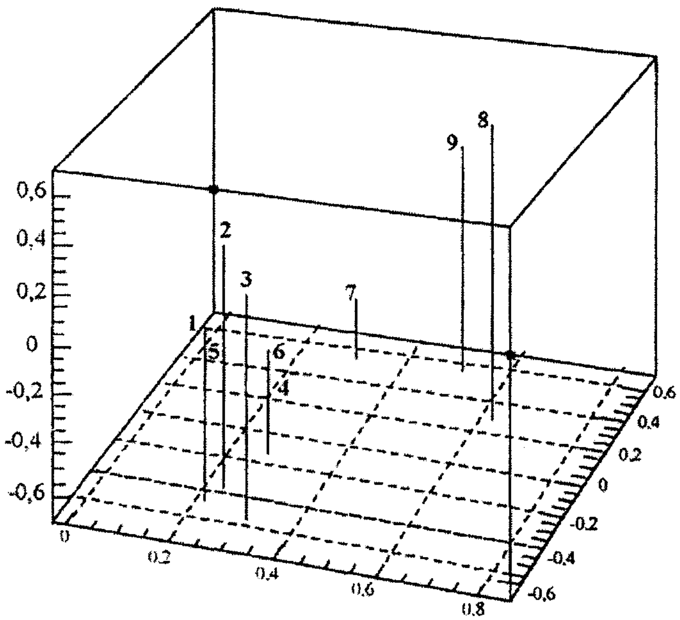

Figure 9 Plot of the first three loading vectors (A_1, A_2, and A_3). (From Ref. 74.)

separation time. Usually, one AMD run consists of 10–30 separate development steps, each 1–3 mm longer than the previous step. Between two developments, the plates are dried in vacuum to remove the solvent, and the mobile phase is removed also from the chromatographic chamber. These steps are repeated until the entire developing program is completed. In each chromatographic run, the bottom part of the spot starts to migrate while the top part does not move, so the spot is reconcentrated and the diffusion effect that usually controls the chromatographic separation is strongly decreased. Thus, the spots will be focused as bands of 0.1–1 mm width, depending on the compound characteristics.

The optimum AMD separation is that in which all components are separated from each other and the spots are distributed along the length of the layer. The peak positions on the final chromatogram depend on the choice of mobile-phase composition and the shape of the gradient, and correct adjustment of the several instrumental settings of the AMD equipment is required. Various solvent compositions can be used to form the AMD gradient, and the best choice is usually achieved by empirical experimentation. The gradients used in AMD can be universal gradients that contain a sudden change in the solvent strength or linear gradients that provide a linear change in the solvent strength.

Many authors compared isocratic TLC with AMD, concluding that the number of separated compounds is greater with AMD than with isocratic TLC and that chromatographic separation is optimized with AMD (78,79). Because AMD is an instrumental technique, it can be coupled online with other chromatographic methods, and this represents a new trend in chromatographic analysis. For example, Stan and Schwarzer (80) realized the on-line coupling of reversed-phase HPLC with AMD on a normal-phase layer. This coupling represents a very promising technique because it allows the combination of two different separation principles.

AMD is suitable for the separation of multicomponent mixtures in TLC and is a useful tool that provides more powerful screening than conventional TLC methods. This technique provides large spot capacities because the reconcentration effect is caused by multiple development as well as by the accommodation of many spots on the same chromatographic plate due to gradient development. Moreover, reproducibility, separation quality, and the possibility to obtain accurate and reproducible quantitative determination have been significantly improved by using the AMD technique.