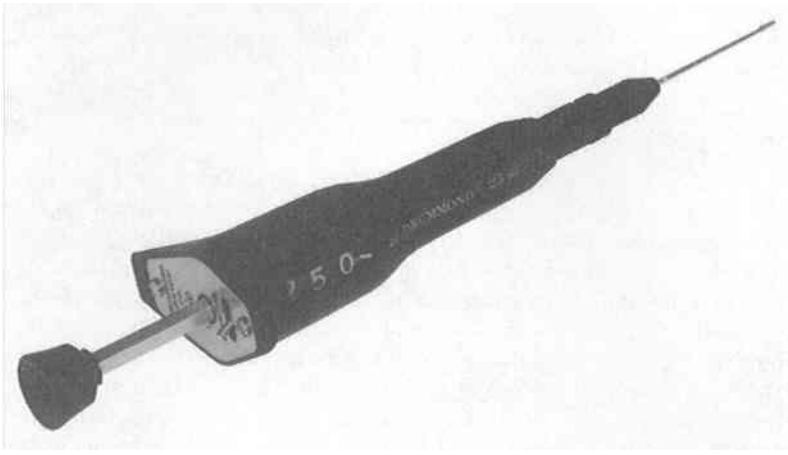

Figure 4 Twenty-five microliter digital microdispenser. (Photograph supplied by Drummond Scientific.)

as spots or bands in any order to specified layer positions through a capillary pipet that is rinsed with solvent between applications.

C. Formation of Bands

Bands or streaks of sample are applied manually, are applied automatically with the Camag Linomat, are formed automatically during development by use of plates with a preadsorbent or concentrating area (see Sec. IV.B), or are produced by predevelopment on conventional plates. Manual application essentially involves placing a contiguous series of spots from a syringe or micropipet side by side. Even with practice, it is difficult to do this uniformly and reproducibly on a conventional plate. The Linomat, which is based on movement of the plate underneath a fixed syringe from which a nitrogen atomizer sprays the sample onto the origin at a constant rate, is advantageous because larger sample volumes [40 μL or more (127)] can be concentrated during the application process compared to other HPTLC devices, and variable volumes of the same standard solution can be applied for calibration in densitometry.

In using preadsorbent plates, samples are spotted or streaked onto the preadsorbent area, and narrow, accurately aligned, homogeneous bands form automatically at the interface with the sorbent upon development. When laned or channeled plates are used, the length of the band is confined within the channel. Sample application is fast and simple for relatively large volumes (up to ~50 μL for TLC and 25 μL for HPTLC). High efficiency can be obtained for HPTLC by spotting larger volumes of dilute solutions rather than nanoliter volumes of highly concentrated solutions. Crude samples can be directly spotted, and salts and other impurities may be retained in the preadsorbent and not interfere with sample resolution or detection. Figure 5 shows the sequence of zone separation in various stages of development on a preadsorbent TLC plate.

The final method for forming initial bands is to concentrate a large spot into a line by partial predevelopment of the layer with a strong solvent in which all components move with the solvent front. After the plate is dried, it is developed with the mobile phase needed to provide resolution.

It has been shown that bands give sharper separations and lower detection limits than spots and are advantageous for densitometry because the length of the scanner light beam can be made shorter than the length of the band (one-half to two-thirds of the original band length). This method of aliquot scanning minimizes the need to exactly position the zone within the beam.

D. Sample Application for Preparative Layer Chromatography

The amount of sample that can be applied to a preparative layer depends on the mixture to be separated, the layer, and the development method. Adsorption TLC permits the application of