

may be available from other manufacturers and have been made in the laboratory. The presence of a fluorescent indicator facilitates nondestructive detection. The term "micropreparative layer chromatography (MPLC)" has been used for separations of up to 10 mg on a 0.25 mm analytical layer.

Samples are applied as streaks, either manually or with an instrument such as the Linomat, or by using a solid-phase sample application (SPSA) device (149). Ascending and horizontal development have been used most often, and the mobile phase is often chosen after preliminary tests on analytical plates. Incompletely separated bands are scraped and eluted and rechromatographed on a second plate (262). Multiple development and gradient elution have been applied for complex mixtures (263), and RPC (Fig. 2) and OPLC have also been used for PLC and MPLC. A recent application of centrifugal PLC is the fractionation of moderate molecular weight polysiloxanes for use as secondary standards for column gel permeation chromatography (GPC) (263a).

If the compounds to be recovered are not colored or fluorescent and do not absorb UV light, a detection reagent must be applied to the edges of the plate to locate the zones to be recovered. Plates with prescored edges facilitate this process. Pure compounds are recovered by scraping and elution with a suitable solvent (Fig. 9).

Layers for PLC are discussed in Section IV.G, and application of zones in Section V.D.

Procedures and apparatus for PLC are described in Chapter 12 of Ref. 1, in Chapter 11 of this Handbook, and in Ref. 149.

XII. RADIOCHEMICAL TECHNIQUES

Radio-TLC techniques are described in Chapter 12 of this Handbook and Chapter 13 of Ref. 1.

Thin-layer radiochromatography (TLRC), or radio-TLC, is used for separation, identification, and measurement of radioisotopes. The principal methods employed are autoradiography, zonal analysis, and the use of radiation detectors. An important application of TLRC is in the quality control and development of radiopharmaceuticals such as Tc-99m (264).

In autoradiography, an X-ray or photographic film is exposed to emissions from radioactive zones on the layer to produce an image on the film. The exposed film is developed by the usual photographic methods to reveal spots of varying darkness at the locations of the separated zones. The darkness, which is related to the amount of radioactivity, can be quantified by densitometry using a calibration curve prepared from a film exposed to zones of radioactive standards. A disadvantage is the long exposure period required for certain weakly radioactive isotopes. Two variations of direct-exposure autoradiography are direct exposure with an intensifying screen (plates coated with inorganic phosphors) and fluorography (impregnation of a scintillator into the layer followed by direct exposure).

For zonal analysis, zones are removed by scraping, the sorbent is transferred into vials, scintillation fluid is added, and the light emitted due to interaction of the radioactive nuclei with the fluid is measured with a scintillation counter. The study of bile acids in humans by liquid scintillation counting coupled with densitometry is an example of an application (265).

A variety of radiation detectors have been used for TLRC, including spark chambers, radioscaners, linear analyzers, a radioanalytic imaging system (Wallac Ambis), multiwire proportional counters, and phosphor imaging analyzers. All of these have been described in Chapter 12 of the first and second editions of this Handbook, but use of the latter two has been reported most often since the second edition was published and is discussed below.

The DAR is a two-dimensional position-sensitive multiwire proportional counter that measures all radioactive zones simultaneously on a 20 × 20 cm plate. The metabolism of the anxiolytic compound deramciclone was studied by using DAR after separation by conventional TLC (266) and in combination with TLC/FAB-MS-MS (219) and OPCL/MS (218,267,268). TLC and DAR were also coupled for the analysis of neutral C₁₄ lipids neosynthesized by the human sebaceous gland (269).

Phosphor or bioimaging analyzers operate by using a phosphor imaging plate made of fine crystals of BaF:Eu²⁺ (Fugi Photo Co., Ltd.) to store emitted beta energy from the layer; scanning