

PRINCIPLES OF INSTRUMENTAL ANALYSIS

FIFTH EDITION

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PRINCIPLES OF Instrumental Analysis

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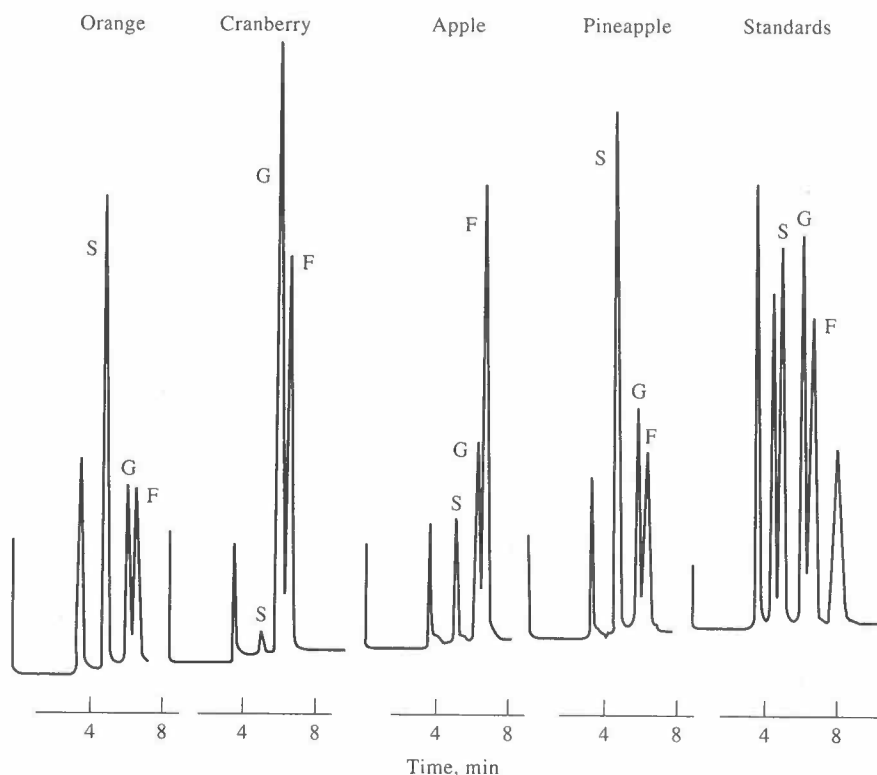


Figure 28-29 Determination of glucose (G), fructose (F), and sucrose (S) in canned juices. (Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)

The disadvantages are: (1) only a limited number of bands can be accommodated because the time scale of the chromatogram is short; and (2) inapplicability to samples of similar size, such as isomers. Generally, a 10% difference in molecular weight is required for reasonable resolution.

28H THIN-LAYER CHROMATOGRAPHY

Planar chromatographic methods include *thin-layer chromatography* (TLC) and *paper chromatography* (PC), and *electrochromatography*. Each makes use of a flat, relatively thin layer of material that is either self-supporting or is coated on a glass, plastic, or metal surface. The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or an electrical potential. Planar chromatography is sometimes called two-dimensional chromatography, although this description is not strictly correct inasmuch as the stationary phase does have a finite thickness.

Currently, most planar chromatography is based upon the thin-layer technique, which is faster, has bet-

ter resolution, and is more sensitive than its paper counterpart. This section is devoted to thin-layer methods only.

28H-1 The Scope of Thin-Layer Chromatography

In terms of theory, the types of stationary and mobile phases, and applications, thin-layer and column liquid chromatography are remarkably similar. In fact, an important use of thin-layer chromatography, and the reason this topic is included here, is to serve as a guide to the development of optimal conditions for performing separations by column liquid chromatography. The advantages of following this procedure are the speed and low cost of the exploratory thin-layer experiments. Some chromatographers, in fact, take the position that thin-layer experiments should always precede column experiments.

In addition to its use in developing column chromatographic methods, thin-layer chromatography has

become the workhorse of the drug industry for the all-important determination of product purity. It has also found widespread use in clinical laboratories and is the backbone of many biochemical and biological studies. Finally, it finds widespread use in the industrial laboratories.⁴¹ As a consequence of these many areas of application, it has been estimated that at least as many analyses are performed by thin-layer chromatography as by high-performance column liquid chromatography.⁴²

28H-2 How Thin-Layer Separations Are Performed

Typical thin-layer separations are performed on flat glass or plastic plates that are coated with a thin and adherent layer of finely divided particles; this layer constitutes the stationary phase. The particles are similar to those described in the discussion of adsorption, normal- and reversed-phase partition, ion-exchange, and size-exclusion column chromatography. Mobile phases are also similar to those employed in high-performance column liquid chromatography.

Thin-Layer Plates

Thin-layer plates are available from several commercial sources at costs that range from \$1 to \$10 per plate. The common plate sizes in centimeters are 5×20 , 10×20 , and 20×20 . Commercial plates come in two categories, conventional and high-performance. The former have thicker layers (200 to 250 μm) of particles having nominal particle sizes of 20 μm or greater. High-performance plates usually have film thicknesses of 100 μm and particle diameters of 5 μm or less. High-performance plates, as their name implies, provide sharper separations in shorter times. Thus, a conventional plate typically will exhibit 2000 theoretical plates in 12 cm with a development time of 25 min. The corresponding figures for a high-performance plate are 4000 theoretic-

cal plates in 3 cm requiring 10 min for development. High-performance plates suffer from the disadvantage of having a significantly smaller sample capacity.

Sample Application

Sample application is perhaps the most critical aspect of thin-layer chromatography, particularly for quantitative measurements. Usually the sample, as a 0.01% to 0.1% solution, is applied as a spot 1 to 2 cm from the edge of the plate. For best separation efficiency, the spot should have a minimal diameter—about 5 mm for qualitative work and smaller for quantitative analysis. For dilute solutions, three or four repetitive applications are used, with drying in between.

Manual application of samples is performed by touching a capillary tube containing the sample to the plate or by use of a hypodermic syringe. A number of mechanical dispensers, which increase the precision and accuracy of sample application, are now offered commercially.

Plate Development

Plate development is the process in which a sample is carried through the stationary phase by a mobile phase; it is analogous to elution in liquid chromatography. The most common way of developing a plate is to place a drop of the sample near one edge of the plate and mark its position with a pencil. After the sample solvent has evaporated, the plate is placed in a closed container saturated with vapors of the developing solvent. One end of the plate is immersed in the developing solvent, with care being taken to avoid direct contact between the sample and the developer (Figure 28-30). The developing solvent travels up the plate, being drawn by capillary action between the fine particles. As the developing solvent travels past the point of sample application, it dissolves

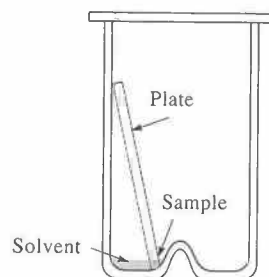


Figure 28-30 A typical development chamber.

⁴¹ Monographs devoted to the principles and applications of thin-layer chromatography include: R. Hamilton and S. Hamilton, *Thin Layer Chromatography*. New York: Wiley, 1987; B. Fried and J. Sherma, *Thin-Layer Chromatography*, 3rd ed. New York: Marcel Dekker, 1994; J. C. Touchstone, *Practice of Thin-Layer Chromatography*, 2nd ed. New York: Wiley, 1983. For briefer reviews, see D. C. Fenimore and C. M. Davis, *Anal. Chem.*, **1981**, *53*, 253A; S. J. Costanzo, *J. Chem. Educ.*, **1984**, *61*, 1015; C. F. Poole and S. K. Poole, *Anal. Chem.*, **1989**, *61*, 1257A.

⁴² T. H. Mauch II, *Science*, **1982**, *216*, 161; J. C. Touchstone, *LC-GC*, **1993**, *11*(6), 404.