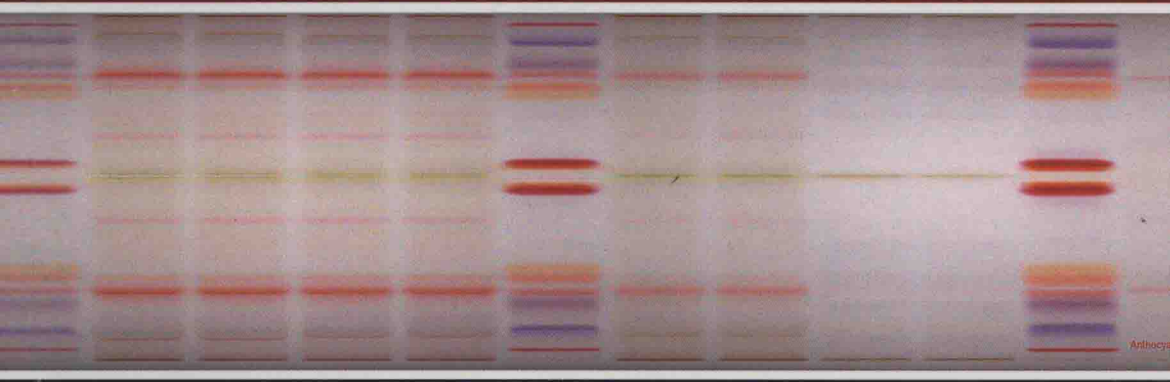
Colin Poole

Instrumental Thin-Layer Chromatography



INSTRUMENTAL THIN-LAYER CHROMATOGRAPHY

Edited by

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CHAPTER

1

Milestones, Core Concepts, and Contrasts

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1.1 INTRODUCTION

Column chromatography and thin-layer chromatography are alternative formats for liquid chromatography [1]. Both formats exist as simple laboratory tools requiring little instrumentation and also as fully instrumental techniques. In both the cases, the stationary phase consists of a sorbent bed containing homogeneously packed particles or as a porous monolith. When movement of the mobile phase through the sorbent bed is controlled by capillary forces, the separation performance is suboptimal but requires little instrumentation affording a convenient and flexible arrangement for simple separations at the analytical or preparative scale. For faster separations, or separations with a higher peak capacity, a mechanism is required to enhance the mobile phase velocity. This requires instrumentation to pressurize the mobile phase and is the basis of high-pressure (or high-performance) liquid chromatography (HPLC) for columns and forced flow (or overpressured layer chromatography) for layers [2–4]. Although forced-flow instrumentation for thin-layer chromatography is commercially available, it is not in common use. Thus, whereas the practice of HPLC is a forced-flow technique, the practice of thin-layer chromatography is predominantly a capillary-controlled flow technique. In the latter case, instrumentation is required to optimize the various steps in the separation process and is referred to as high-performance thin-layer chromatography (HPTLC), or instrumental thin-layer chromatography, to distinguish the technique from conventional thin-layer chromatography (TLC) performed with much simpler equipment [5,6]. The general advantages of utilizing HPLC conditions versus conventional column chromatography are well known. The same argument cannot be made for

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conventional TLC versus HPTLC, and the general migration of separations from conventional TLC practices to HPTLC has not been universal. In fact, one might say that conventional TLC thrives in the laboratory environment as a quick, inexpensive, flexible, and portable method for surveying the composition of simple mixtures while only a few laboratories are equipped to perform more complex and quantitative analyses by HPTLC.

1.2 MILESTONES

The origins of thin-layer chromatography can be traced to the experiments on drop chromatography performed by Izmailov and Shraiber in the late 1930s [7]. From this beginning, thin-layer chromatography evolved into a fast and more powerful tool than gravity flow column chromatography for analytical separations. Thin-layer chromatography, as we know it today, was established in the 1950s due in large part to the efforts of Stahl and Kirchner on different continents. Their main contribution was the development of standardized materials and procedures that led to improved performance and reproducibility, as well as popularizing the technique by contributing many new applications [8]. At about the same time, commercialization of materials and devices commenced making the technique accessible to all laboratories. This ushered in the golden era of thin-layer chromatography where it quickly displaced paper chromatography as the main analytical liquid chromatographic method. By the 1970s, high-pressure liquid chromatography was becoming firmly established as an alternative approach for liquid chromatography and eventually grew to eclipse thin-layer chromatography for analytical applications. Thin-layer chromatography did not disappear in subsequent years but became less well known to those working in analytical laboratories where its strengths were often under appreciated. Developments continued in thin-layer chromatography as indicated by the time line Figure 1.1 [6,9].

First the development of high-performance thin-layer chromatography in the late 1970s is discussed. Layers coated with smaller particles of a narrow size distribution required the development of instruments for their convenient use. This was achieved by the early 1980s and so began the second era of thin-layer chromatography, known as modern or instrumental thin-layer chromatography. The evolutionary changes during this second era are captured in a series of books, which if ordered chronologically, represent the state-of-the-art at different times during this period to the present [5,10–15]. The main characteristic features of modern thin-layer chromatography are the use of fine particle layers for fast and efficient separations; sorbents with a wide range of sorption

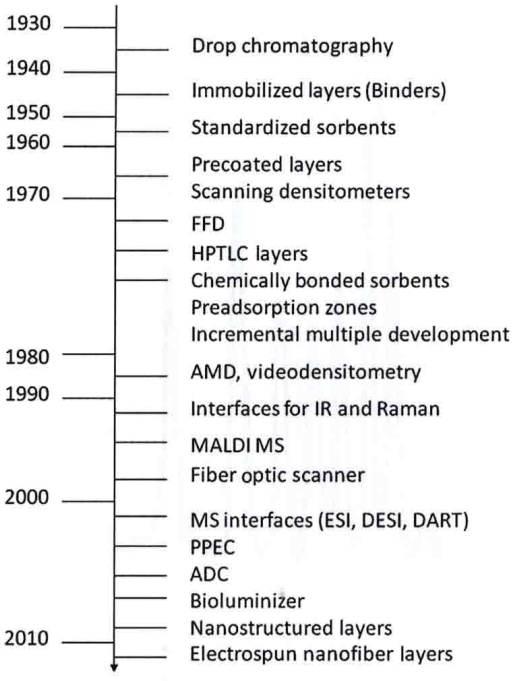

FIGURE 1.1 Time line depicting important developments in the evolution of modern thin-layer chromatography. FFD = forced-flow development in an overpressured development chamber; AMD = automated multiple development chamber; AMC = automatic development chamber; and PPEC = pressurized planar electrochromatography.

properties to optimize selectivity; the use of instrumentation for convenient and usually automated sample application, development and detection; and the accurate and precise in situ recording and quantification of chromatograms. Improvements in virtually all aspects of thin-layer chromatography continued over the next quarter century as indicated in Figure 1.1 and form the basis of subsequent chapters in this book. This period also marks the beginning of the philosophical division between conventional and high-performance thin-layer chromatography that has not been crossed by all those who use thin-layer chromatography. Expectations in terms of performance, ease of use, and quantitative information from the two approaches to thin-layer chromatography are truly opposite (see Section 1.1). As an example of expectations for a separation by modern thin-layer chromatography, see the chromatogram in Figure 1.2 for structurally similar ethyl estrogens (steroids used for birth control) [2]. Because of the small structural differences for these compounds, a high selectivity is required for their separation. Baseline separation is obtained with a short migration distance typical of fine particle layers and scanning densitometry provides a conventional record

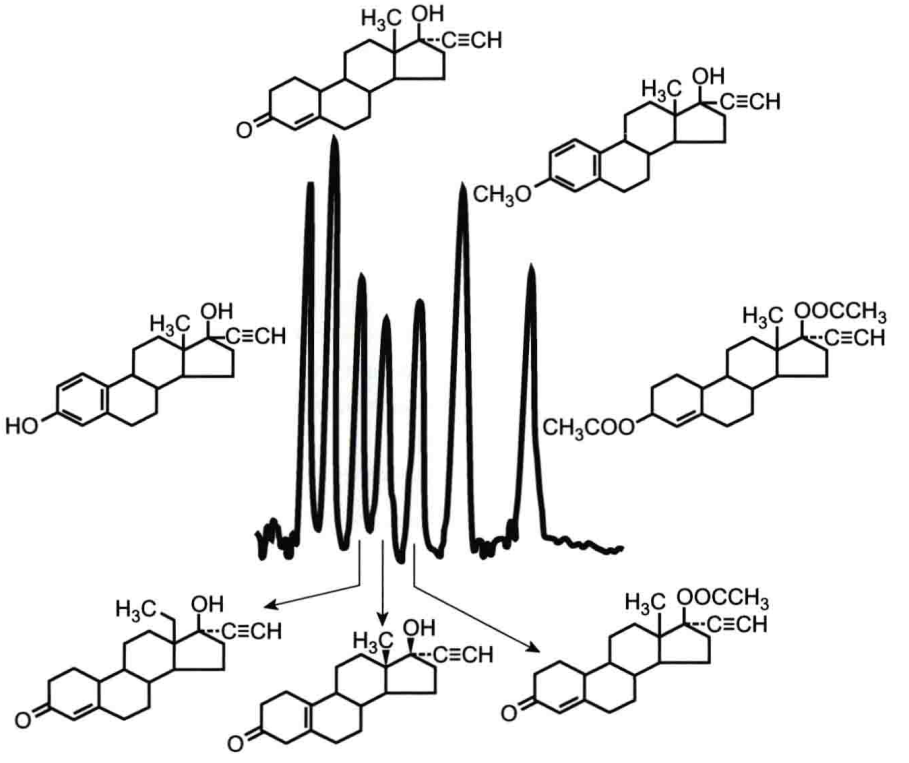

FIGURE 1.2 Separation of ethynyl steroids by modern thin-layer chromatography. Two 15 min developments with the mobile phase hexane–chloroform–carbon tetrachloride–ethanol (7:18:22:1) on a silica gel HPTLC plate. The chromatogram was recorded by scanning densitometry at 220 nm. *Reproduced with permission from Ref. [2].*

of the separation in the form of a chromatogram, as well as quantification of individual steroids after calibration. The quantitative results for tablet analysis are as accurate and precise as other chromatographic methods and the method is suitable for high-throughput routine tablet conformity analysis in which sample preparation requires no more than dissolution and filtration. Some specific reasons for choosing thin-layer chromatography for quantitative analysis are outlined in the next section.

1.3 ATTRIBUTES OF A PLANAR FORMAT

Columns afford a better arrangement for operation at high pressures and for variation of the separation conditions by the control of external parameters. The thin-layer format provides a better arrangement for high sample throughput, flexible detection strategies, and a greater tolerance of samples with a high-matrix burden [2,16]. The throughput advantage is a consequence of the possibility of separating multiple samples in parallel

with each sample occupying a single lane (or track) on the layer and several samples assigned to different lanes for simultaneous separation. Column chromatography is inherently a sequential separation process in which the separation time for a group of samples is the product of the number of samples and the cycle time for an individual separation. A single 10 × 10 cm HPTLC plate can separate 18 samples and standards simultaneously if developed in one direction and twice that number if developed from two opposing edges to the center.

Separations in columns employ the elution mode in which all sample components experience the same separation distance defined by the column length, but because of the different nature of their interactions with the stationary phase, are separated in time. The typical experimental arrangement employs an injection device to insert the sample into the pressurized mobile phase close to the column entrance and an online detector at the column exit to record the separation [1]. For planar chromatography, it is more common to use a variation of the elution mode for the separation known as development. In this case, the mobile phase moves through the layer in a definite direction for a fixed distance, which is usually less than the bed length in the direction of mobile phase migration. In contrast to column chromatography individual sample components are separated in space (have different migration distances) achieved in an identical separation time. This has additional consequences for detection. Sample components are detected in the stationary phase, compared to the mobile phase in column chromatography, and at the completion of the separation the separated zones are stationary and can be interrogated free of time constraints. This simplifies the use of chemical and biological reagents for detection. A growing application of thin-layer chromatography is in effect-directed analysis where a biological response from, for example, luminescent bacteria or enzyme inhibition, is used to indicate the presence of substances with a specific toxic mechanism in contrast to structure-based detection strategies that are the basis of most common dynamic detection techniques [17,18]. In the development mode, the whole sample is contained in the sample lane and is available for detection. This is important for determining the integrity of a sample in contrast to column chromatography where the only sample components observed are those that are fully eluted from the column. Planar chromatography can be used to screen samples to predict their behavior in column chromatography and to assess the need for sample cleanup.

Typical applications in thin-layer chromatography are one-use applications after which the stationary phase (layer) is discarded. On account of their higher cost columns for HPLC are used to analyze multiple samples. Effective sample preparation improves the quality of information for target compounds in complex mixtures by both thin-layer and column chromatography, but a difference between the two techniques is

TABLE 1.1 Attributes of a Planar Format for Liquid Chromatography

Attribute	Application
Separation of samples in parallel	Low-cost analysis and high-throughput screening of samples requiring minimal sample preparation.
Disposable stationary phase	Analysis of crude samples (minimizing sample preparation requirements) analysis of a single or small number of samples when their composition and/or matrix properties are unknown. Analysis of samples containing components that remain sorbed to the separation medium or contain suspended microparticles.
Static detection	Samples requiring postchromatographic treatment for detection samples requiring sequential detection techniques (free of time constraints) for identification or confirmation separations can be evaluated in different locations or at different times.
Sample integrity	Total sample occupies the chromatogram not just that portion of the sample that elutes from the column.

the capability of thin-layer chromatography to handle samples with a heavy matrix burden without the need of potentially extensive remedial action. It is important that the sample contains no strongly retained components or suspended particles for column chromatography as these may alter the properties of the column and affect its ability to separate further samples. This is not a concern for thin-layer chromatography because reuse of the stationary phase is not planned, and therefore, in the initial phases of a study designed to gain an understanding of the properties of the sample matrix on the analysis, or to facilitate the analysis of samples with minimal sample preparation, then thin-layer chromatography is the method of choice [2,16].

The attributes of the planar format that are the basis for the continuing use of thin-layer chromatography in liquid chromatography are summarized in Table 1.1. The application chapters in this book provide definitive examples where these advantages have been realized in practice. In our own studies, we have preferred thin-layer chromatography for the screening step to identify samples requiring a detailed analysis (target analyte possibly present in a small number of samples), for samples with a heavy matrix burden or unknown matrix properties, for effect-directed analysis (target analytes unknown), for class fractionation (identification of analytes by group membership rather than individual identity), and for the standardization of plant materials by fingerprint analysis.

For some sample types, we generally prefer column chromatography, or use thin-layer chromatography for initial screening and column chromatography for the analysis. Samples that require a high peak capacity for their separation and identification are usually better handled