

9.2.3.3 The Chromatogram

Differential Chromatogram

A chromatogram obtained with a differential detector (see Fig. 9.2.1.A).

Integral Chromatogram

A chromatogram obtained with an integral detector (see Fig. 9.2.1.B).

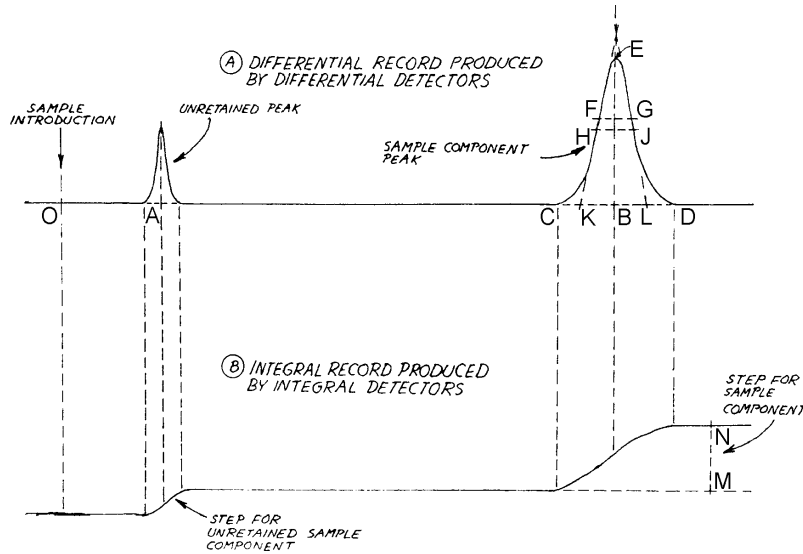


Figure 9.2.1 Typical chromatogram: A, differential record produced by differential detector; B, integral record produced by integral detector.

Starting Point or Line

The point or line on a chromatographic paper or layer where the substance to be chromatographed is applied (*P* in Fig. 9.2.2).

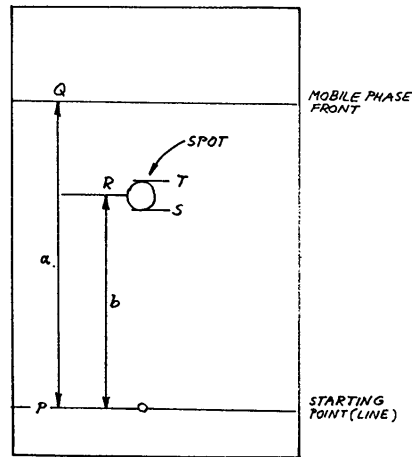


Figure 9.2.2 Typical planar chromatogram

Spot

A zone in paper and thin-layer chromatography of approximately circular appearance.

Spot Diameter (ST in Fig. 9.2.2)

The width of the sample component spot before or after chromatography.

Baseline

The portion of the chromatogram recording the detector response when only the mobile phase emerges from the column.

Peak

The portion of a differential chromatogram recording the detector response when a single component is eluted from the column (see Fig. 9.2.1.A). If separation is incomplete, two or more components may be eluted as one *Unresolved Peak*.

Peak Base (CD in Fig. 9.2.1.A)

The interpolation of the baseline between the extremities of the peak.

Peak Area (CHFEGJD in Fig. 9.2.1.A)

The area enclosed between the peak and the peak base.

Peak Maximum (E in Fig. 9.2.1.A)

The point on the peak at which the distance to the peak base, measured in a direction parallel to the axis representing detector response, is a maximum.

Peak Height (EB in Fig. 9.2.1.A)

The distance between the peak maximum and the peak base, measured in a direction parallel to the axis representing detector response.

Standard Deviation (σ)

The term in the exponent of the equation relating the width and height of a Gaussian peak:

$$y = y_0 \exp\left[-\frac{x^2}{2\sigma^2}\right]$$

where y is the peak height at any point on the peak, y_0 is the peak height at maximum, x is the distance from the ordinate (i.e., half of the width at that point), and σ is the standard deviation of the peak. In practice, the standard deviation can be calculated from one of the peak-width values specified below.

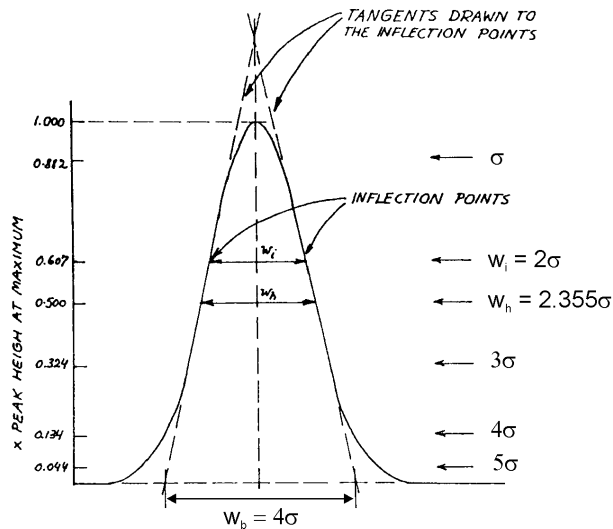


Figure 9.2.3 Widths of a Gaussian peak at various heights, as a function of the standard deviation of the peak.

Variance of the Peak

The square of the standard deviation (σ^2).

Peak-Widths

Peak-widths represent retention dimensions (time or volume) parallel to the baseline. If the baseline is not parallel to the axis representing time or volume, then the peak-widths

are to be drawn parallel to this axis. Three peak-width values are commonly used in chromatography (see Fig. 9.2.1.A and Fig. 9.2.3).

In the case of Gaussian (symmetrical) peaks, the peak-widths are related to the standard deviation (σ) of the peak according to the following equations:

$$w_b = 4\sigma$$

$$w_h = 2\sigma\sqrt{2\ln 2} = 2.355\sigma$$

$$w_i = 2\sigma$$

Peak-Width at Base (w_b) (KL in Fig. 9.2.1.A and Fig. 9.2.3)

The segment of the peak base intercepted by the tangents drawn to the inflection points on either side of the peak.

Peak-Width at Half Height (w_h) (HJ in Fig. 9.2.1.A and Fig. 9.2.3)

The length of the line parallel to the peak base at 50% of the peak height that terminates at the intersection with the two limbs of the peak.

Note: The peak-width at base (w_b) may be called the "base width". However, the peak width at half height (w_h) must never be called the "half width" because that has a completely different meaning. Also, the symbol $w_{1/2}$ should never be used instead of w_h .

Peak-Width at Inflection Points (w_i) (FG in Fig. 9.2.1.A and Fig. 9.2.3)

The length of the line drawn between the inflection points parallel to the peak base.

Tailing

Asymmetry of a peak such that, relative to the baseline, the front is steeper than the rear. In paper chromatography and thin-layer chromatography, it refers to the distortion of a spot showing a diffuse region behind the spot in the direction of flow.

Fronting

Asymmetry of a peak such that, relative to the baseline, the front is less steep than the rear. In paper chromatography and thin-layer chromatography, it refers to the distortion of a spot, showing a diffuse region in front of the spot in the direction of flow.

Step

The portion of an integral chromatogram recording the amount of a component, or the corresponding change in the signal from the detector as the component emerges from the column (see Fig. 9.2.1.B).

Step Height (NM in Fig. 9.2.1.B)

The distance, measured in the direction of detector response, between straight-line extensions of the baselines on both sides of a step.

Internal Standard

A compound added to a sample in known concentration to facilitate the qualitative identification and/or quantitative determination of the sample components.

External Standard

A compound present in a standard sample of known concentration and volume which is analyzed separately from the unknown sample under identical conditions. It is used to facilitate the qualitative identification and/or quantitative determination of the sample components. The volume of the external standard (standard sample) need not to be known if it is identical to that of the unknown sample.

Marker

A reference substance chromatographed with the sample to assist in identifying the components.