Instruction

parameters

Common & Optimization Parameters

Retardation Factor (R_F) - The ratio of the distance traveled by the substance's spot center to the total distance traveled by the solvent front (eluent). It is the primary retention value in planar chromatography (TLC), always ranging from 0 to 1.

$$R_F = \frac{a}{b}$$

Capacity Factor (k) - It measures how much longer a substance is retained by the stationary phase compared to the time it spends in the mobile phase.

$$k = \frac{1 - R_F}{R_F}$$

Legend

a: distance traveled by the substance (in cm)

b: distance traveled by the mobile phase (in cm)

Common & Optimization Parameters

Logarithmic Retardation Factor (RM_{Log10}) - A logarithmic transformation of the capacity factor (k), calculated to linearize retention data and is often used to study structure-retention relationships (QSRR).

$$R_M = \log \frac{1 - R_F}{R_F} = \log k$$

Average Retention Factor ($\bar{R}_F$) - The arithmetic or weighted mean of the Rf values for the substance from a series of replicates.

$$\overline{R_F} = \frac{\sum_{i=1}^{m} (R_F)_i}{m}$$

Legend

For **Average Retention Factor** m = 2 & both values are RF of neighbour spots

Common & Optimization Parameters

Separation factor (α) - The ratio of the retention factors (k) of two consecutively eluting components (k2 /k1), a measure of phase selectivity. α>1 is required for separation.

$$\alpha = \frac{k_2}{k_1} \quad \text{where } k_2 > k_1$$

k1: Retardation Factor of the one substance in pair

k2: Retardation Factor of the other substance in pair

Pair of neighbour substances

Common & Optimization Parameters

Tailing factor (T_F) - A measure of peak asymmetry, defined as the ratio of the sum of the distances from the peak center to the front and back edges (at 5% or 10% height) to twice the distance from the front edge to the center. A value of 1 indicates no tailing (perfect symmetry).

$$A_s = \frac{B}{A}$$

Measured at **10%** of the peak height.

Asymmetry factor (As) - A measure of the peak's symmetry; the ratio of the distance from the midpoint to the end (tail) of the peak to the distance from the start (front) to the midpoint, measured at a specific fraction of the peak height (e.g., 10%). A value of **1** indicates perfect symmetry.

$$T_f = \frac{A + B}{2A}$$

Measured at **5%** of the peak height.

Resolution Parameters

$$RS = 0.25 \left[\frac{k_2}{k_1} - 1 \right] \cdot \sqrt{\bar{R}_F \cdot N} \cdot (1 - \bar{R}_F)$$

Selectivity Efficiency Retention

Efficiency - European Pharmacopeia - The metric for the quality of separation, often equivalent to the Number of Theoretical Plates (N), calculated using the European Pharmacopeia formula.

(European Pharmacopeia)

Efficiency - United States Pharmacopeia - The efficiency value calculated using the United States Pharmacopeia formula.

(United States Pharmacopeia)

Selectivity - A measure of the chromatographic system's (stationary and mobile phases) ability to differentiate between the separated components. Equivalent to the **SEPARATION FACTOR** (α).

Retention - (1 – Mean of RF factors of neighbour spots/peaks).

Resolution Parameters

Resolution (Rs) - European Pharmacopeia -A measure of the degree of separation of two adjacent peaks; calculated based on the difference in peak center distances and their widths, using the European Pharmacopeia formula. Rs ≥1.5 is typically required for complete separation.

$$RS = 0.25 \left[\frac{k_2}{k_1} - 1 \right] \cdot \sqrt{\bar{R}_F \cdot N} \cdot (1 - \bar{R}_F)$$

N – Number of Theoretical plates - (European Pharmacopeia)

Resolution (Rs) - United States Pharmacopeia - The resolution value calculated using the United States Pharmacopeia formula.

$$RS = 0.25 \left[\frac{k_2}{k_1} - 1 \right] \cdot \sqrt{\bar{R}_F \cdot N} \cdot (1 - \bar{R}_F)$$

N – Number of Theoretical plates - (United States Pharmacopeia)

Resolution Parameters

Resolution (Rs) - universal Formula - The general measure of the degree of separation of two adjacent peaks, often calculated according to universally used Formula.

$$\mathbf{R_S} = \frac{z_2 - z_1}{0.5(w_1 + w_2)}$$

Legend

z1 and z2 - Distance of the spot centers from the sample application point (the distance of the **peak maxima** on the densitogram). z2 corresponds to the component that traveled further.

w1 and w2 - Width of the spots measured along the direction of chromatogram development (the **width of the base** of the peaks obtained on the densitogram).

Theoretical plates

$$\mathbf{H_{obs}} = \frac{w_{\text{base}}^2}{16 z_x} = \frac{w_{0.5}^2}{5.54 z_x}$$

United States Pharmacopeia **European Pharmacopeia**

Height equivalent to a theoretical plate (HETP) - European Pharmacopeia - The average column/track length over which a single equilibrium partitioning process occurs. A smaller value indicates higher efficiency. Calculated using the European Pharmacopeia formula.

Height equivalent to a theoretical plate (HETP) - United States Pharmacopeia - The HETP value calculated using the United States Pharmacopeia formula.

Wbase = Peak width at 0% height

W0.5= Peak width at 50% height

Theoretical plates

Number of theoretical plates (N) - European Pharmacopeia - A measure of separation efficiency. A higher value indicates better efficiency. Calculated using the European Pharmacopeia formula.

$$\mathbf{N_{obs}} = 16 \left[\frac{z_x}{w_{\text{base}}} \right]$$

Number of theoretical plates (N) - United States Pharmacopeia - The N value calculated using the United States Pharmacopeia formula.

$$\mathbf{N_{obs}} = 5.54 \left[\frac{z_x}{w_{0.5}} \right]^2$$

Theoretical plates

Number of theoretical plates per meter (N/m) - European Pharmacopeia - A measure of efficiency per unit length, using the European Pharmacopeia formula.

$$\mathbf{N/L}_{\text{Ph. Eur.}} = \frac{16}{L}\left[\frac{z_x}{w_{\text{base}}}\right]^2$$

Number of theoretical plates per meter (N/m) - United States Pharmacopeia - The N/m value calculated using the United States Pharmacopeia formula.

$$\mathbf{N/L}_{\text{USP}} = \frac{5.54}{L}\left[\frac{z_x}{w_{0.5}}\right]^2$$

L - Length of the separation path – (pixels per 1 meter)

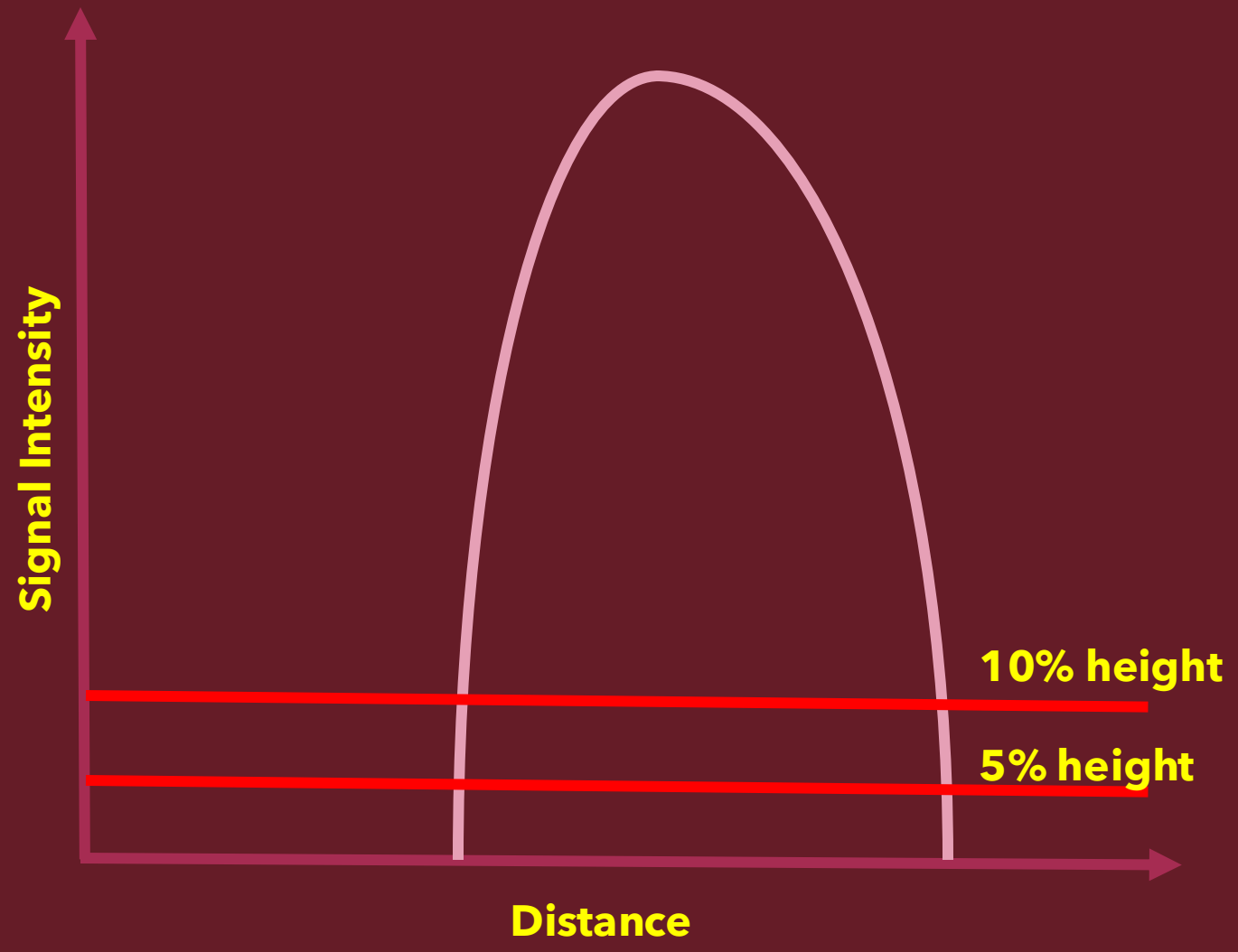
Intermediate Parameters

Startpoint to midpoint distance at 5% of height -The horizontal distance measured from the peak's startpoint to the peak's midpoint, at **5%** of the peak's maximum height.

Startpoint to midpoint distance at 10% of height -The horizontal distance measured from the peak's startpoint to the peak's midpoint, at **10%** of the peak's maximum height.

Midpoint to Endpoint distance at 5% of height - The horizontal distance measured from the peak's endpoint to the peak's midpoint, at **5%** of the peak's maximum height.

Midpoint to Endpoint distance at 10% of height - The horizontal distance measured from the peak's endpoint to the peak's midpoint, at **10%** of the peak's maximum height.



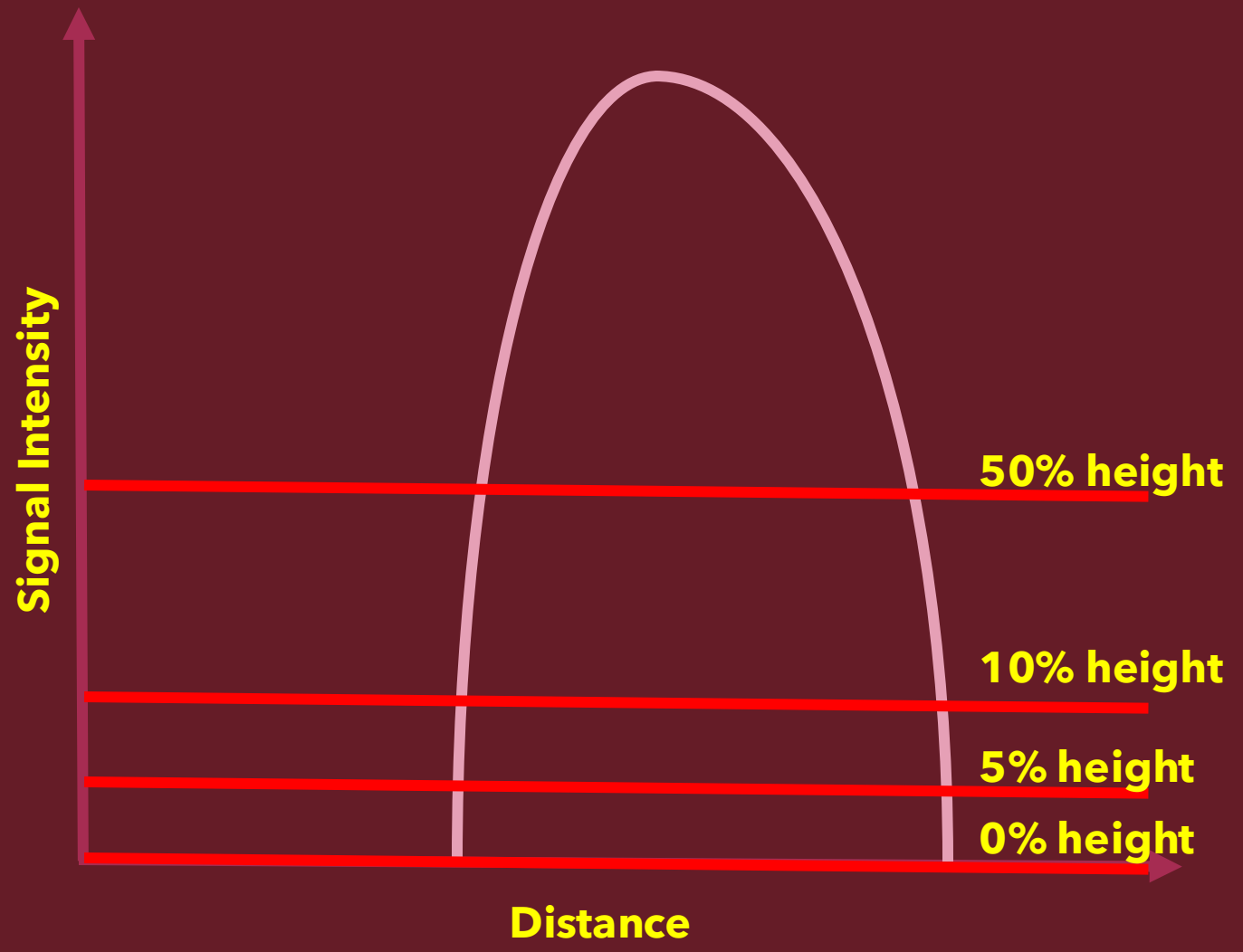
Intermediate Parameters

Peak width at 5% height - The horizontal width of the peak measured at **5%** of its maximum height.

Peak width at 10% height - The horizontal width of the peak measured at **10%** of its maximum height.

Peak width at 50% height - The horizontal width of the peak measured at **50%** of its maximum height.

Peak width at 0% height - The horizontal width of the peak measured at its basis.



Intermediate Parameters

X position of spot center (0-1 range) - The horizontal position of the spot/peak center in plate coordinate system, normalized to the range ⟨0,1⟩.

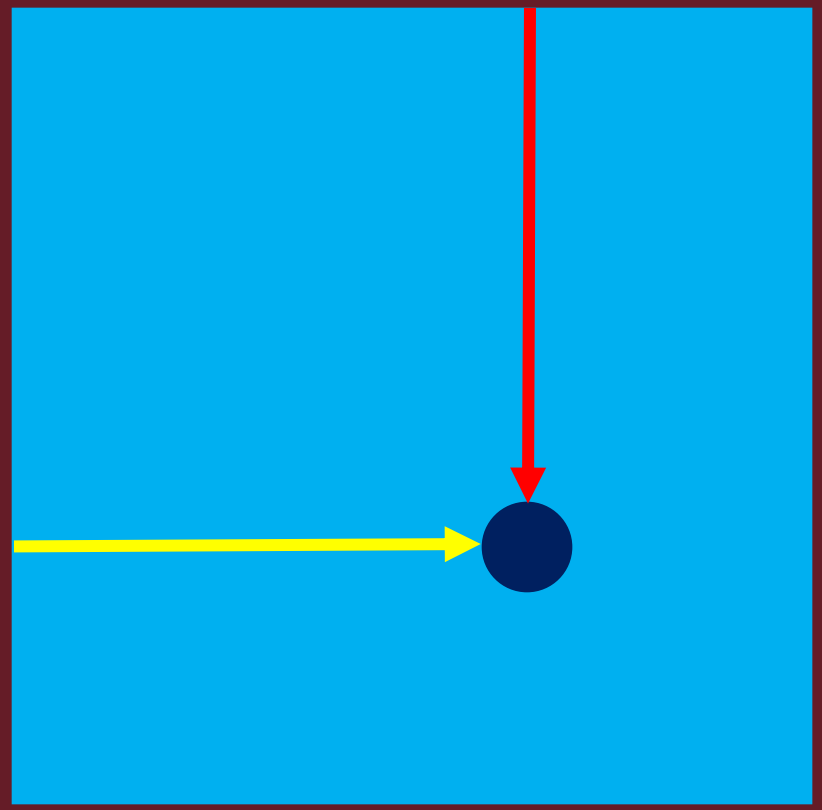
Y position of spot center (0-1 range) - The vertical position of the spot/peak center in plate coordinate system, normalized to the range ⟨0,1⟩.

Midpoint index - The position of the spot/peak center expressed as the index within the track's data array.

Intermediate Parameters

X position of spot center (0-1 range) - The horizontal position of the spot/peak center in plate coordinate system, normalized to the range ⟨0,1⟩.

Y position of spot center (0-1 range) - The vertical position of the spot/peak center in plate coordinate system, normalized to the range ⟨0,1⟩.



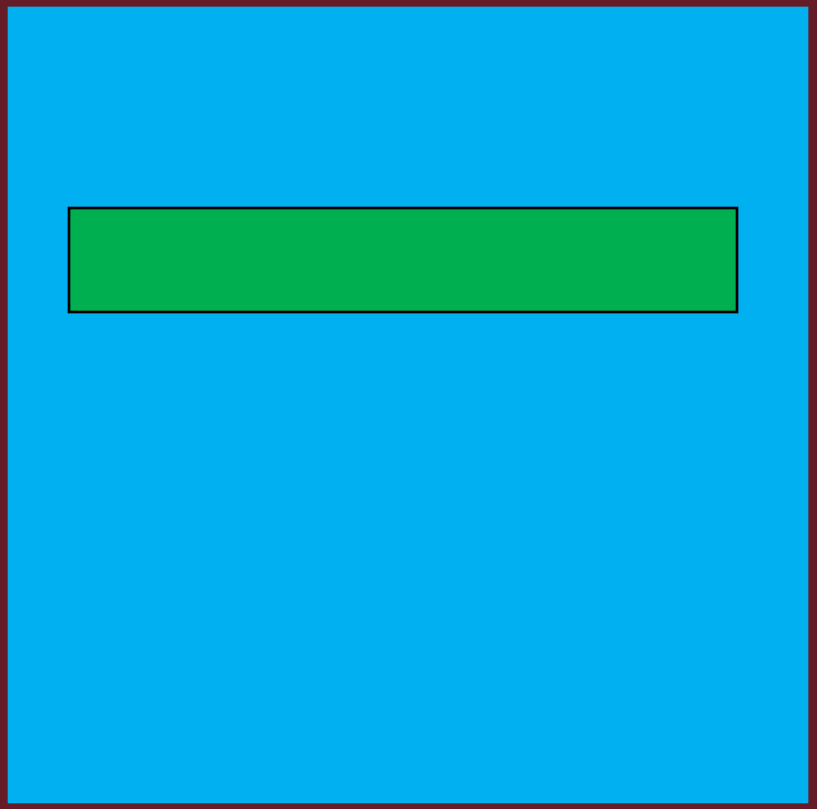
Reference Parameters

Track Name - The name of the chemical compound that has been separated and is being analyzed.

Peak Name - The identifier or column/lane number where the substance's spot/peak was registered.

Plate width in cm - The physical width of the chromatographic plate in centimeters.

Track width in cm - The width of the track to which belongs current peak/spot, expressed in centimeters.



Quantity Parameters

($H_{\%}$) Peak height to Track height ratio - The percentage ratio of the maximal peak height of the substance to the maximal height recorded on the entire track.

(H_{Track}) Maximal height on track - The largest measured intensity/height of any peak across the entire analyzed track.

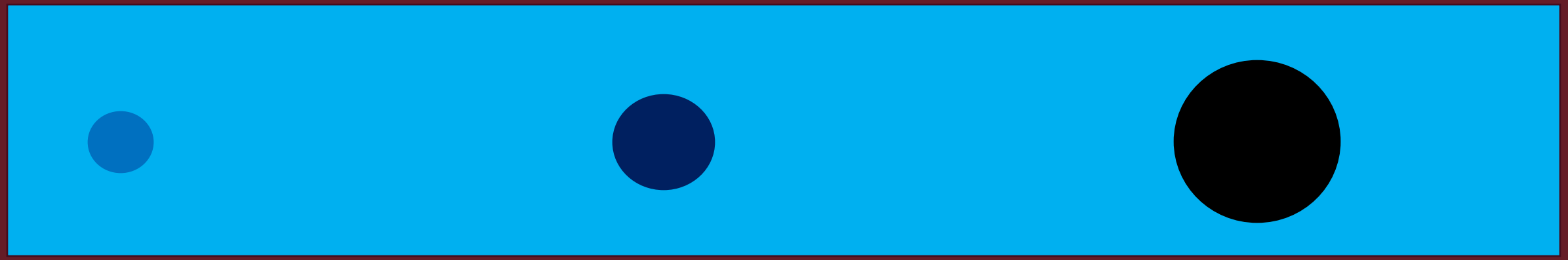
(H_{Max}) Maximal peak height - The greatest measured intensity (height) of the specific peak/spot being analyzed

Quantity Parameters

(S_{Track}) Greatest peak surface for track - The largest measured peak area on the analyzed track, often referring to the main component.

(S%) Peak surface to Track surface ratio - The proportion of the analyzed peak's area relative to the largest peak area on the track, expressed as a percentage. Used to compare spot sizes.

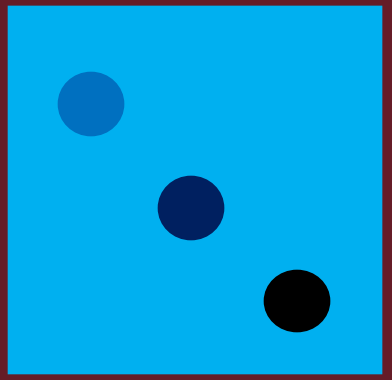
(S) Spot Area - The total area under the peak/spot, often correlated with the quantity of the substance.



Quantity Parameters

Normalized surface - The spot/peak area after normalization (dividing by the estimated surfaces of standard spots in current location of spot on the plate).
!!! Feature is EXPERIMENTAL

Extra curve (like concentration curve) - if impossible to equalize plate illumination digitally



Substance concentration (C) - The estimated or measured quantity (concentration) of the substance within the sample.

Concentration curve formula $y = 0.0001 \cdot x + 9.2479$

Linearity index $R^2 = 1.0000$