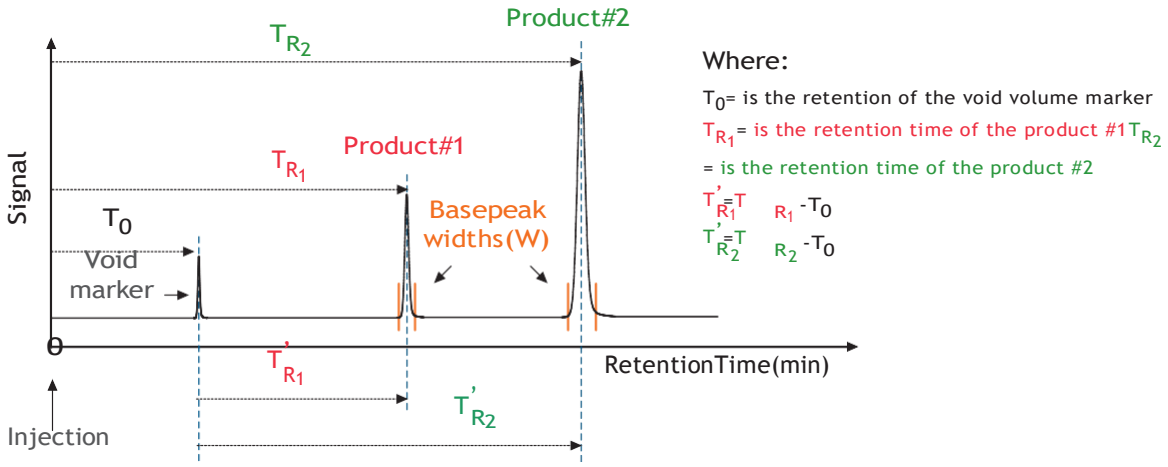
ImportantHPLCDefinitionsandEquations

Typical Chromatogram in liquid chromatography



Capacity Factor or Retention Factor (*k'*) is measured by the retention factor of the analyte compared to an unretained peak (*void volume marker*) using the following equation:

$$k' = \frac{(T_R - T_0)}{T_0}$$

Where:

T_R:is the retention time of the analyte

T_0:is the retention time of the unretained product

Efficiency(*N*) is usually measured by the plate count(*N or all so called theoretical plate number*)using various equations. The most popular ones are:

By USP(*UnitedStatesPharmacopeia*)

$$N = 16 \times \left[\frac{t}{W}\right]^2$$

Where:

N:is the number of theoreticalplates

t:is the retention time of the analyte

W:is the width at the base of the analyte

ByDAB(*GermanPharmacopeia*)

$$N = 5.54 \times \left[\frac{t}{W_{0.5}}\right]^2$$

Where:

N:is the number of theoretical plates

t:is the retention time of the analyte

$W_{0.5}$:is the width-at-half-height of the analyte

Selectivity (a) is measured by the retention factor ratio between two similar compounds.

$$\alpha = \frac{k'_2}{k'_1}$$

Where:

K_1':is the retention factor of product#1

K_2':is the retention factor of product#2

Separation's difficulty based on the selectivity value. If the selectivity is:

≥2: Easy separation
1.5-2: Possible separation*
1.2-1.5: Difficult separation
≤1.2: Very difficult separation**

*Method adjustment could be required
**Selectivity's optimization may be required

Important HPLC Definitions and Equations(*con't*)

Resolution(R) can be expressed using the two following equations

$$R = \frac{\sqrt{N}}{4} \times \left(\frac{\alpha - 1}{\alpha}\right) \times \left(\frac{1 + k'_2}{k'_2}\right)$$

Where:

N:is the number of theoretical plates

a:is the selectivity

K_2':is the retention factor of product#2

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1}$$

Where:

T_1:is the retention time of the product#1

T_2: is the retention time of the product#2

W_1:is the width at the base of the product#1

W_2:is the width at the base of the product #2

Summary of Influencing Factors in HPLC

To chose the most suitable HPLCcolumn ,various parameters need to be taken into account: the desired selectivity and the sample load as well as the efficiency and the resolution. All these parameters are influenced by different factors in HPLC summarized in the table below.

LiquidChromatographyInfluencingFactors

Properties	TypicalParameters	AffectedInfluencingFactors	Limitations
Chromatographic Conditions	Solvent	Retention,Efficiency	Back-pressure&phasestability
	pH	Selectivity,Resolution&Retention	Phasestability
	FlowRate	AnalysisTime,Efficiency&Resolution	Back-pressure&phasestability
Packing Characteristics	Chemistry(*SiO_2,C18,etc.*)	Selectivity,Resolution&Retention	Solventused
	PoreSize (Å)	SampleLoad&Selectivity	Sizeofthe molecule
	ParticleSize (µm)	Back-pressure,Efficiency&Resolution	Back-pressure&flowrate
HPLCColumn Dimensions	InternalDiameter	SampleLoad&Sensitivity	Back-pressure&flowrate
	Length	AnalysisTime&Resolution	Back-pressure&analysistimetoolong

HPLC Method Scaling Up or Scaling Down Theory

When your experimental conditions are well optimized to get the most suitable purification,it is possible to scale up/down your method by keeping the same particle size and sorbent using these two equations:

AdjustmentoftheSampleLoad

$$x_2 = \frac{x_1 \times r_2^2 \times C_L}{r_1^2} \quad \text{where} \quad \left[C_L = \frac{L_2}{L_1}\right]$$

Where:

x_1:is the maximum sample load in initial column

x_2:is the maximum sample load in final column

r_1: is the radius of the initial column

r_2: is the radius of the final column

L_1:is the length of the initial column

L_2: is the length of the final column

AdjustmentoftheFlowRate

$$V_2 = \frac{V_1 \times r_2^2}{r_1^2}$$

Where:

V_1:is the flow rate use with the initialcolumn

V_2: is the flow rate use with the final column

r_1: is the radius of the initial column

r_2:is the radius of the final column

Acceptable Modifications to an HPLCValidated Method

Even if you are using an FDA validated or a USP recommended method,some operating conditions can be adjusted if the modifications respect the acceptable specifications proposed by Pharmacopeias[1-3]and the FDA[4]. A side-by-side comparison of both the original and the adjusted method needs to be performed to demonstrate that the method's accuracy and precision is not affected by these modifications.

AcceptableModificationstoanHPLCValidatedMethod

Parameters	Allowablemodification	Examplesofpossiblemodifications
MobilephasepH	±0.2units	ValidatedpH:7.0 AllowedpHrange:6.8–7.2
Concentrationofsaltsinbuffer	±10%	Validatedconcentration:20mM Allowedconcentrationrange:18-22mM
Ratioofcomponents in mobile phase	Onlytheminorcomponentscanbe adjusted by ± 30% or ± 2% absolute (*i.e.: in regards to the total mobile phase*),whicheveristhelargerbut shouldneverexceed±10%absolute or removed totally.	Binarymixtures: Validated ratio: 50/50 Allowed ratio: 40/60 to 60/40 Validatedratio: 95/5 Allowedratio:93.5/6.5to96.5/3.5 Ternarymixtures: Validatedratio: 60/35/5 Allowed % of the 1st component: 60% Allowed % of the 2nd component: 25 – 45% Allowed % of the 3rd component: 3.5 – 6.5% Thetotalofthethreecomponentstogetherneedtobe100%.
WavelengthofUVdetector	Nomodificationallowed.	n/a
Columnlength	±70%	Validatedlength:150mm Allowedlengthrange:45-255mm
Columninnerdiameter	±50%	Validatedinnerdiameter:4.6mm Allowedinnerdiameterrange:2.3–10.6mm
Flowrate	±50%	Validatedflowrate:1.00mL/min Allowedflowraterange:0.5–1.5mL/min
Injectionvolume	Maybeincreasedtoasmuchas 2 times if no adverse effects on LOD andrepeatability.	n/a
Particlesize	Noincreasepermitted. Maybedecreasedbyasmuchas50%.	Validatedparticlesize:5µm Allowedparticlesizerange:2.5-5µm
Columntemperature	±20%	Validated temperature: 23°CAllowed length range: 18.4 – 27.6°C