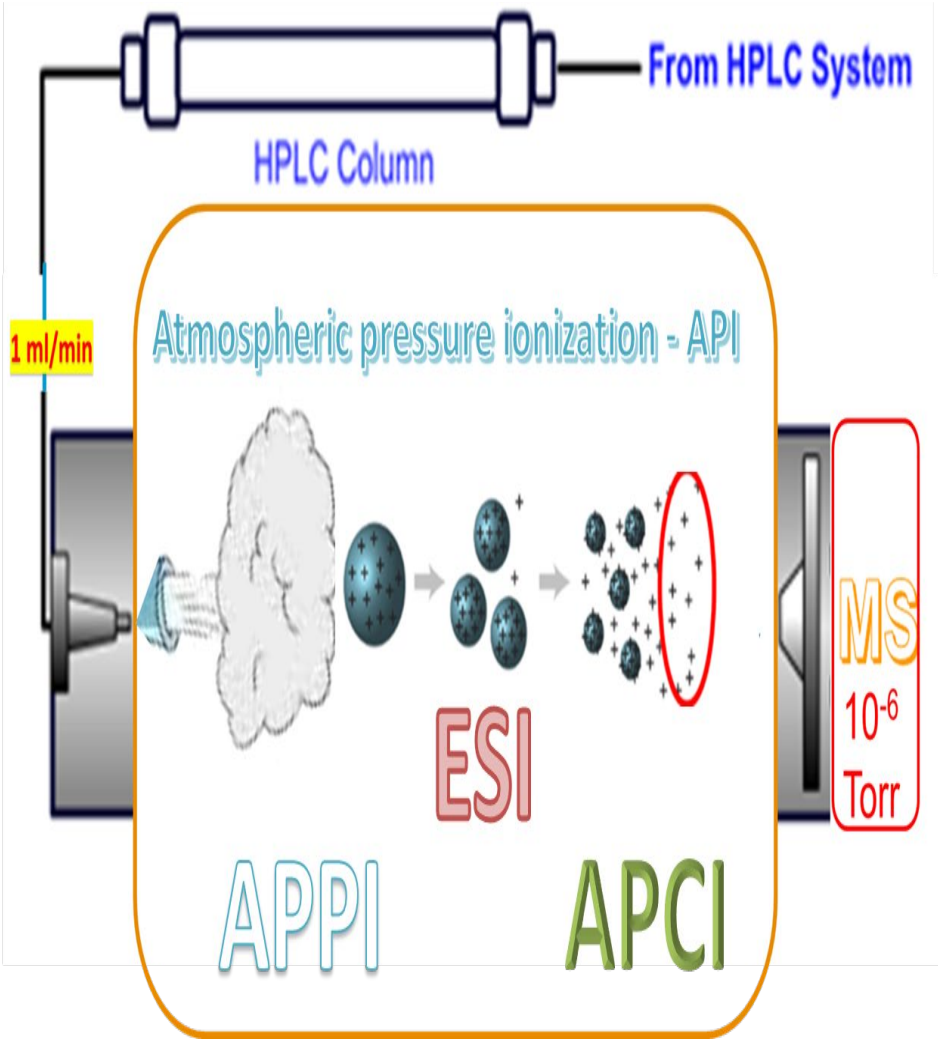
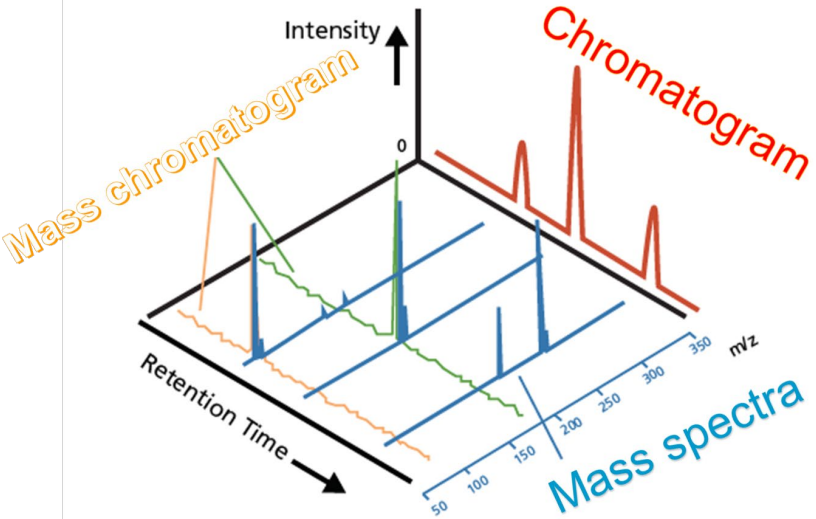
Practical LC-MS method development and bioanalytical method validation

(Formerly "Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS")





Quadrupole

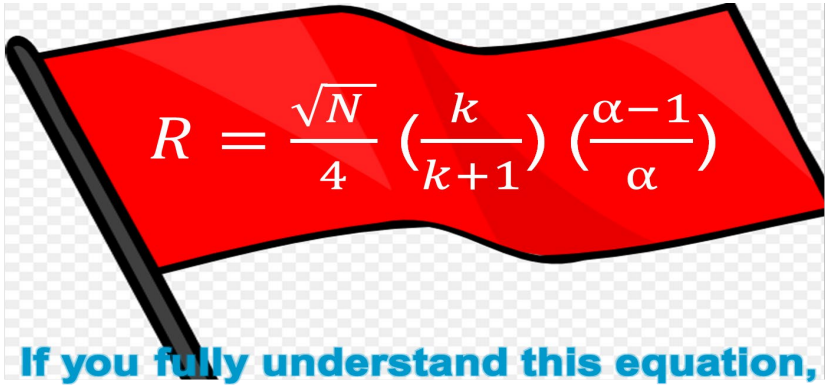
TOF

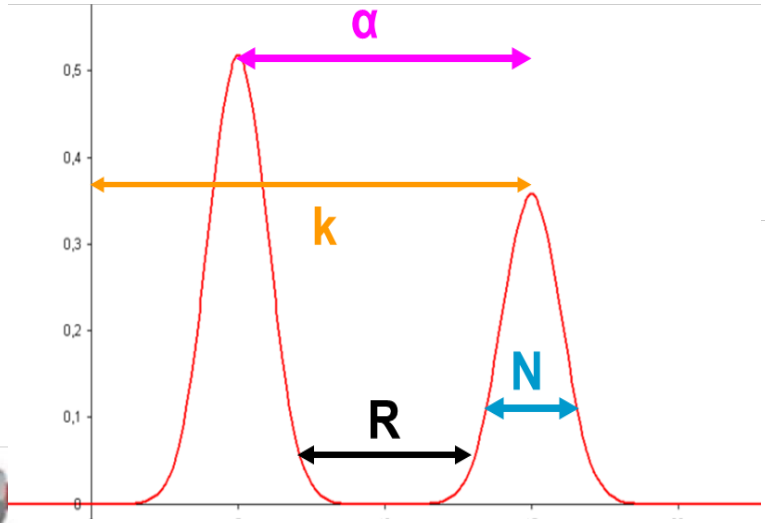
Ion trap

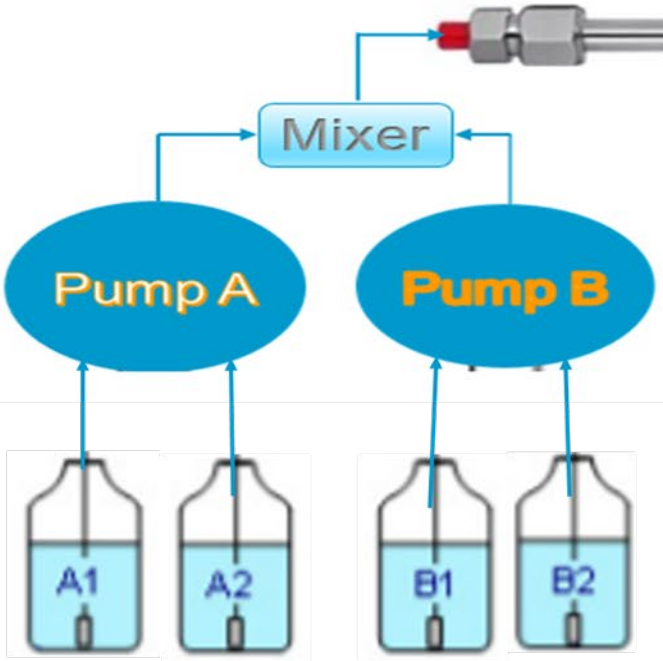
Orbitrap

Practical LC-MS method development and bioanalytical method validation

(Formerly "Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS")



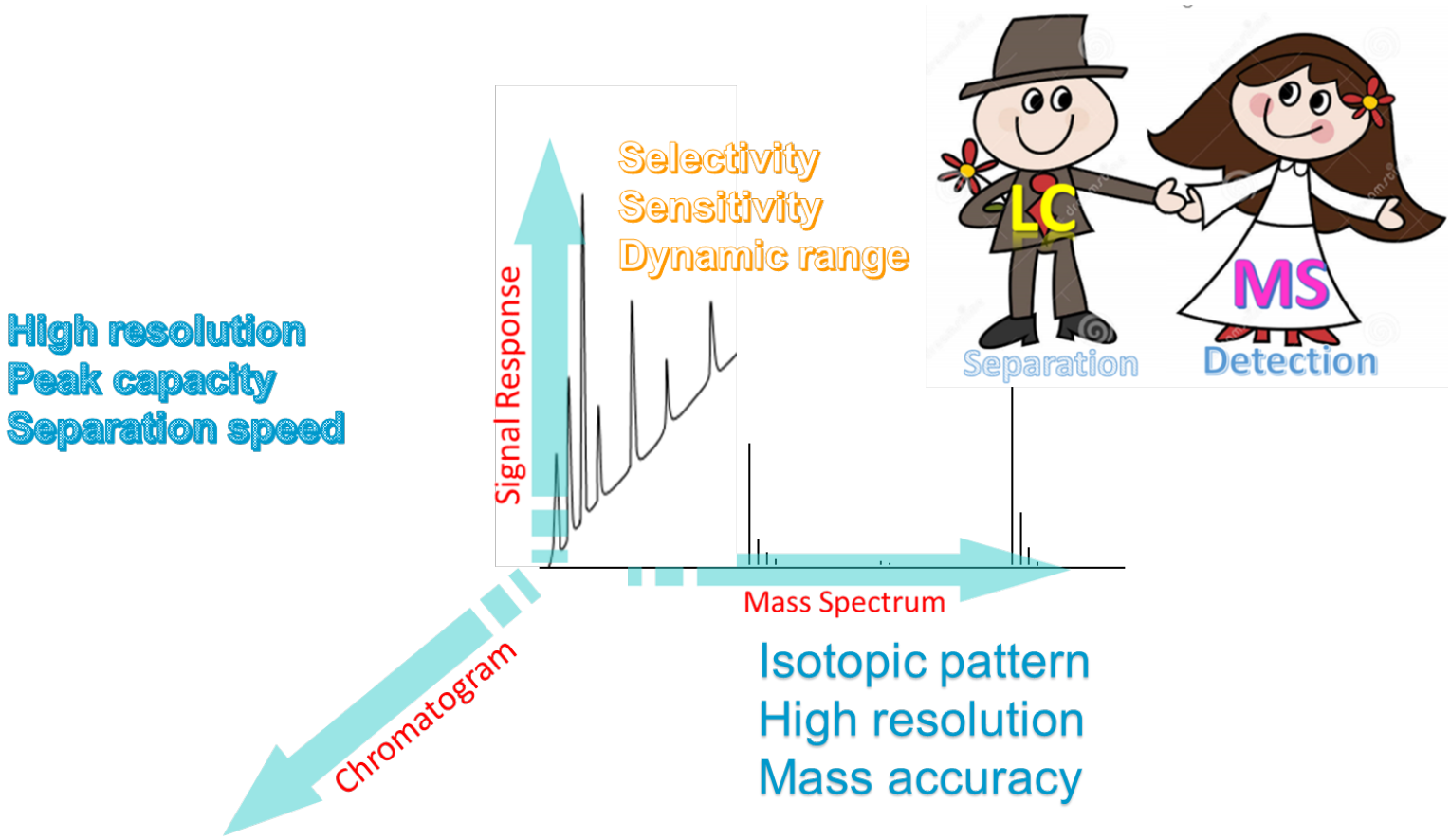




Selectivity (α) impacts the separation most!
unfortunately, it is the most difficult parameter to understand and predict.

Practical LC-MS method development and bioanalytical method validation

(Formerly “Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS”)



Combination of LC with MS – a perfect marriage

Practical LC-MS method development and bioanalytical method validation

(Formerly "Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS")

Validation batch design for **A&P runs**

Prepare 3 runs in different days

Calibration Standards		Quality Control Samples		Other Validation Samples	
Name	Replicate	Name	Replicate	Name	Replicate
Level 1	1	LLOQ	6	Pooled blank plasma	1
Level 2	1	QC1	6	Zero standard	1
Level 3	1	QC2	6	System verification sample	1
Level 4	1	QC3	6		
Level 5	1	QC4	6		
Level 6	1				
Level 7	1				
Level 8	1				
Level 9	1				
Level 10	1				

QC1 ≤ 3 × LLOQ

Accuracy and precision expressions

True = Actual = Theoretical = Nominal value

$$Accuracy = \frac{Determined\ Value}{True\ value} \quad 100 \pm 15\%$$

$Accuracy$ = bias/error/deviation/difference

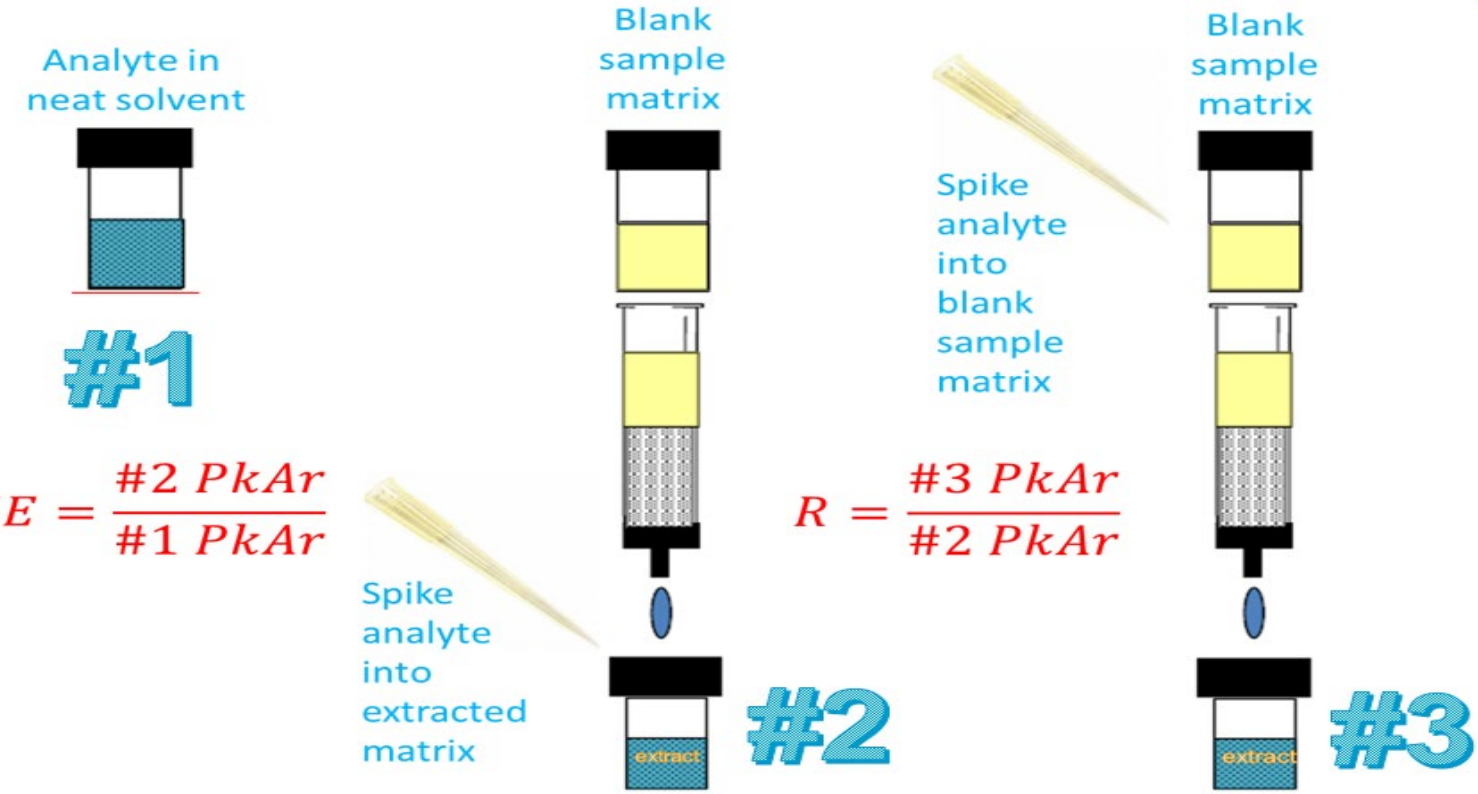
$$\frac{Determined\ Value - True\ Value}{True\ value} \times 100 \quad \pm 15\%$$

$$Precision: RSD = CV\ (coefficient\ of\ variation)$$

Practical LC-MS method development and bioanalytical method validation

(Formerly "Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS")

Matrix effect and extraction recovery



$$ME = \frac{\#2\ PkAr}{\#1\ PkAr}$$

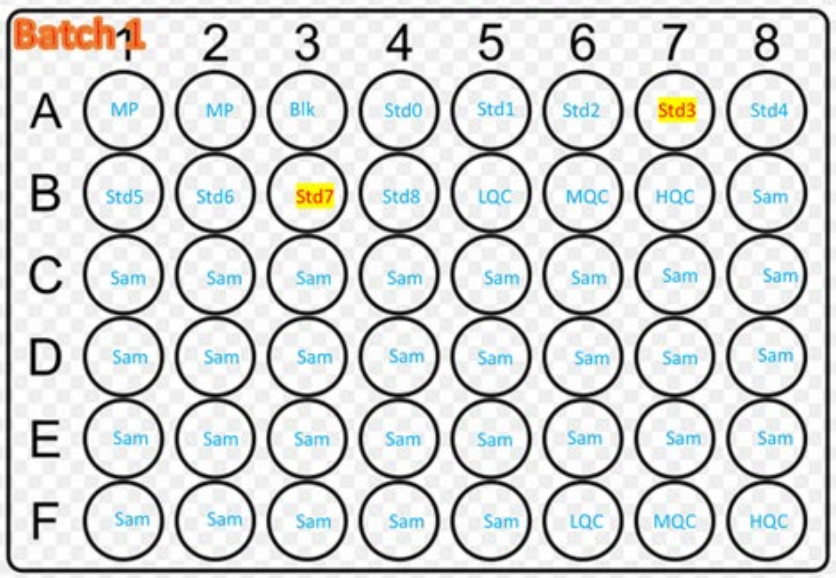
$$R = \frac{\#3\ PkAr}{\#2\ PkAr}$$

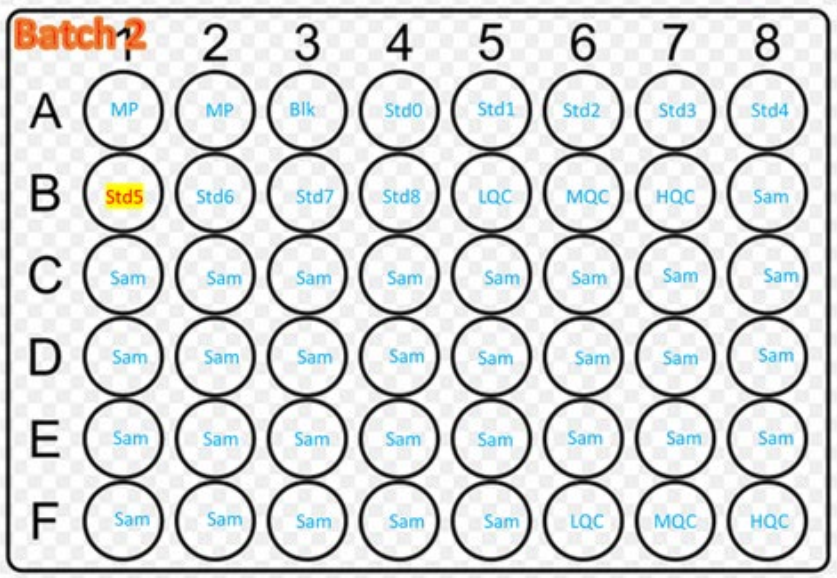
Stable isotopically labeled IS can compensate for extraction recovery and matrix effects

Practical LC-MS method development and bioanalytical method validation

(Formerly "Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS")

Case study (one run = Batch 1 + Batch 2)





Based on 2018 BMV acceptance
75% and a minimum of six standards (6/8 = 75%)
Both batches passed – each batch was processed individually

Practical LC-MS method development and bioanalytical method validation

(Formerly "Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS")

Decision tree for reporting re-assay results