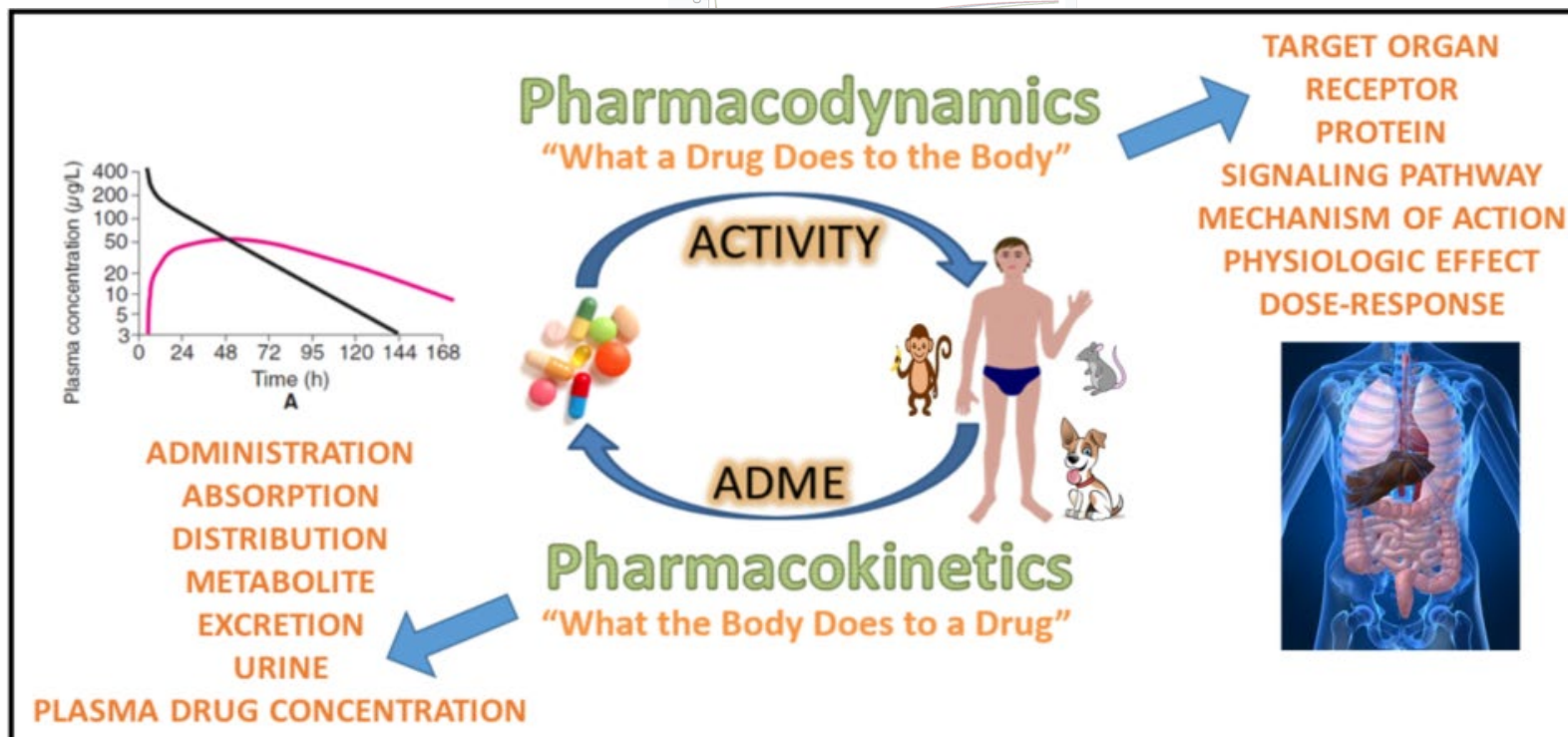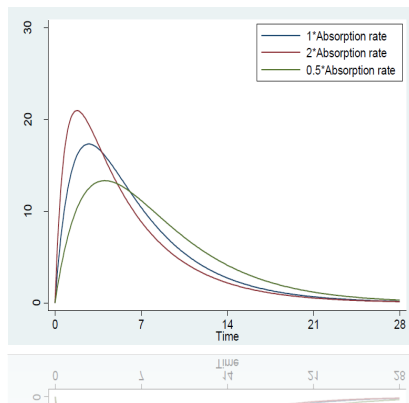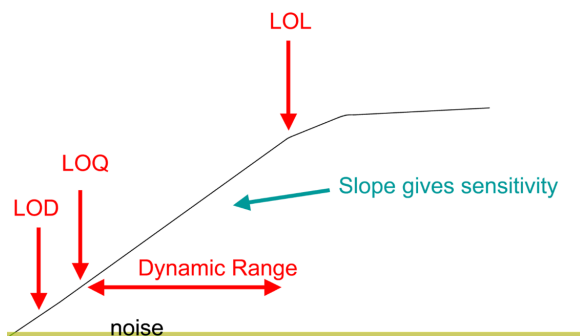# Practical Bioanalytical Method Validation by LC-MS/MS
(Formerly "**Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS**")

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

$$Accuracy = \frac{Determined\ Value}{True\ value} \qquad 100 \pm 15\%$$
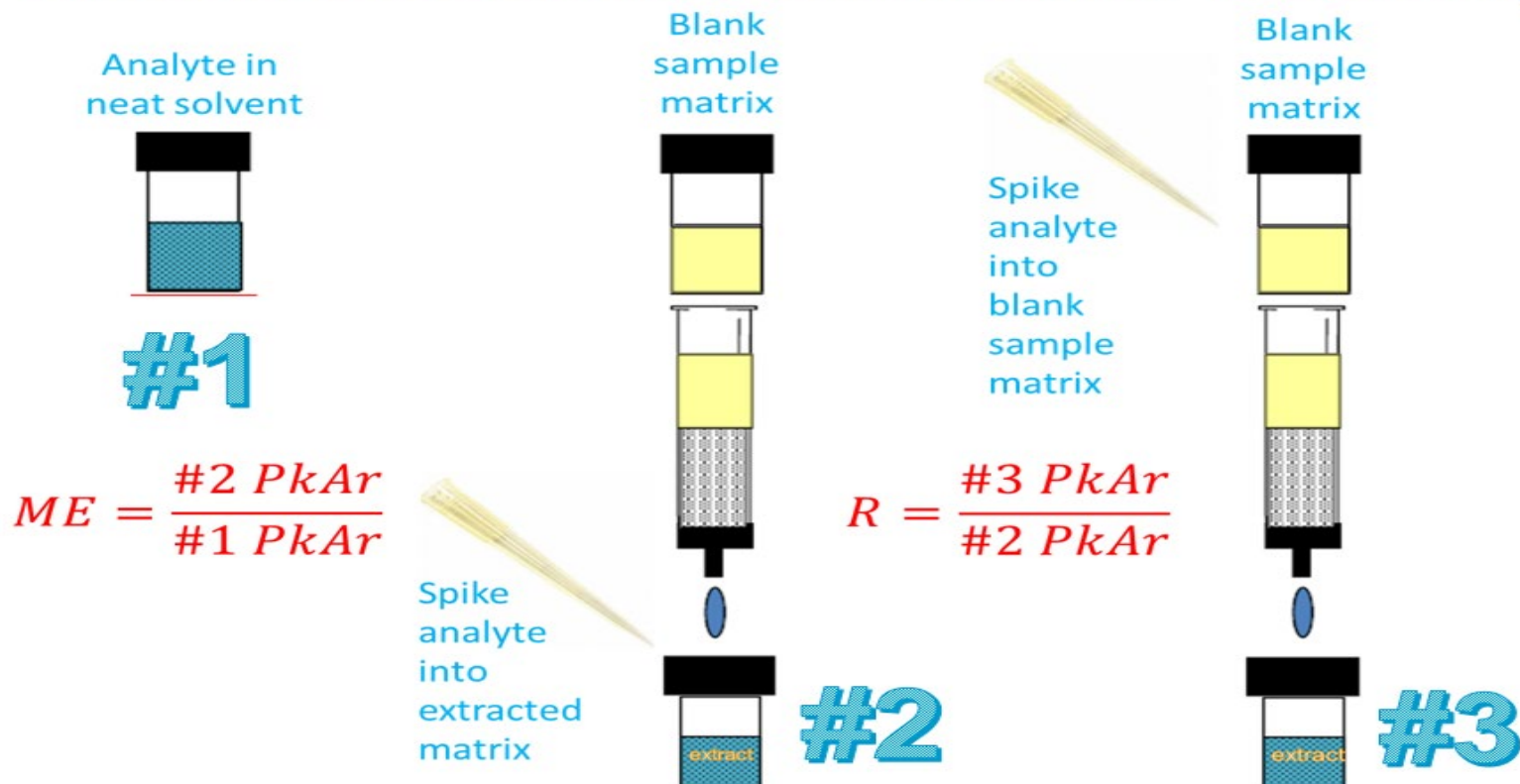
$$Accuracy = bias/error/deviation/difference$$

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

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

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Matrix effect and extraction recovery

Analyte in neat solvent — **#1**

Blank sample matrix — **#2**

Spike analyte into blank sample matrix

Blank sample matrix — **#3**

Spike analyte into extracted matrix

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

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

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

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Case study (one run = Batch 1 + Batch 2)
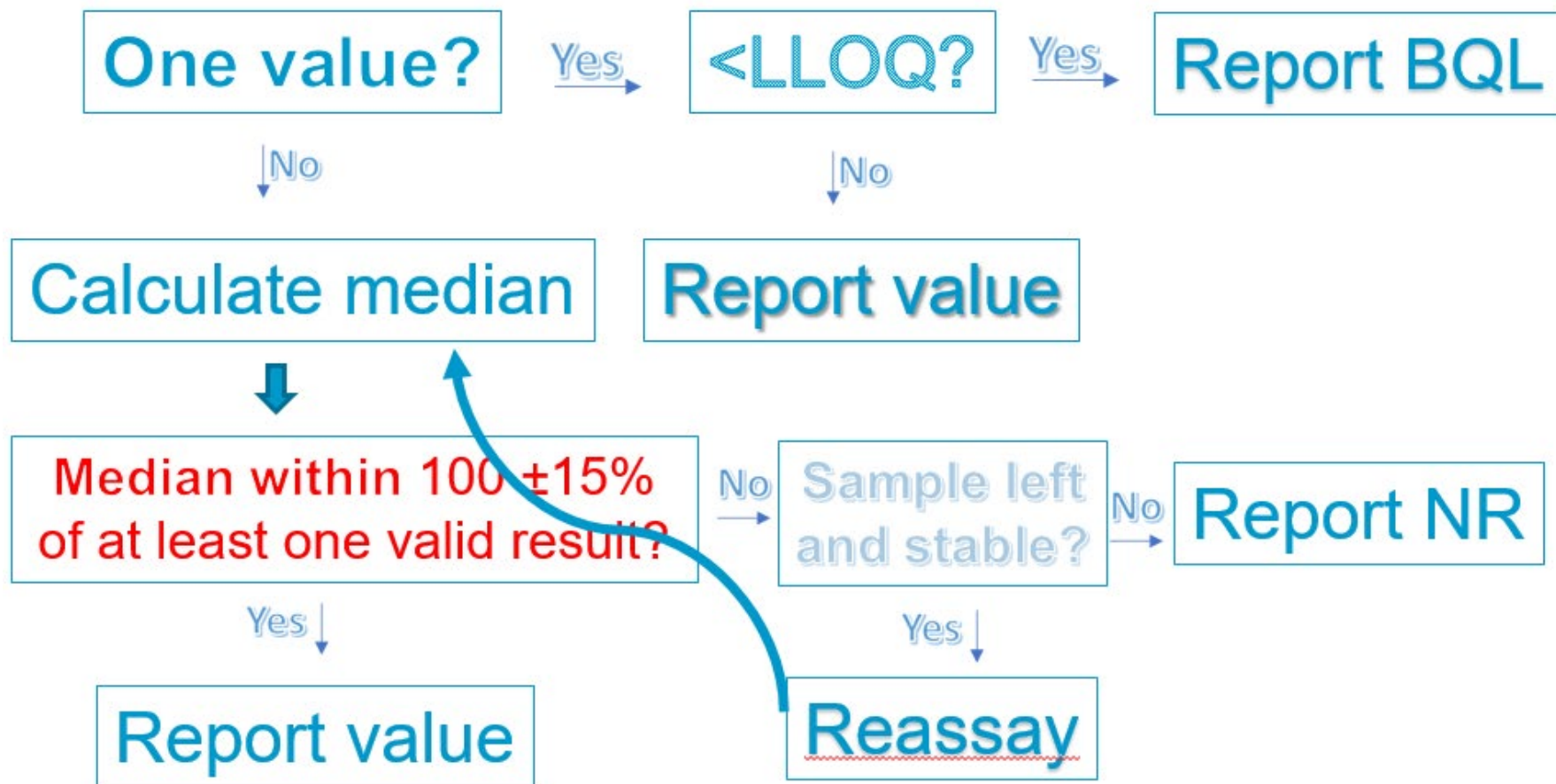
Based on 2018 BMV acceptance 75% **and** a minimum of six standards (6/8 = 75%) Both batches passed – each batch was processed **individually** ✓

Decision tree for reporting re-assay results

Practical Bioanalytical Method Validation by LC-MS/MS
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"Pure" Research → Disease Discovery → Drug Discovery → Preclinical Toxicology → Clinical Tries I, II, III → CMC
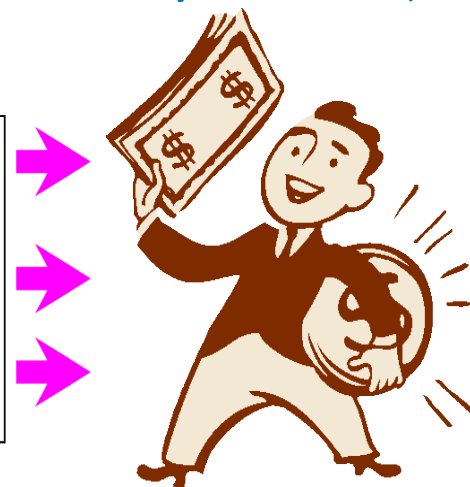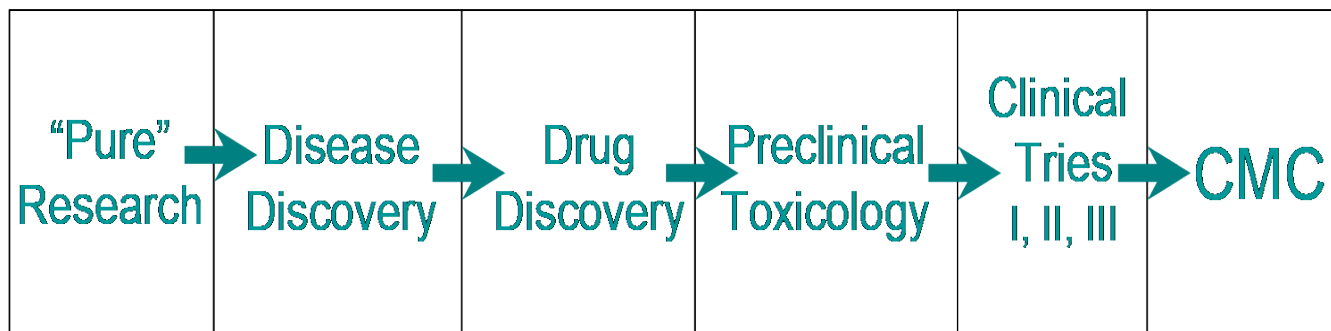
GLP   GCP   GMP

Not regulated

Regulated

Study based   Process based

Good Regulatory Practices (GXP)