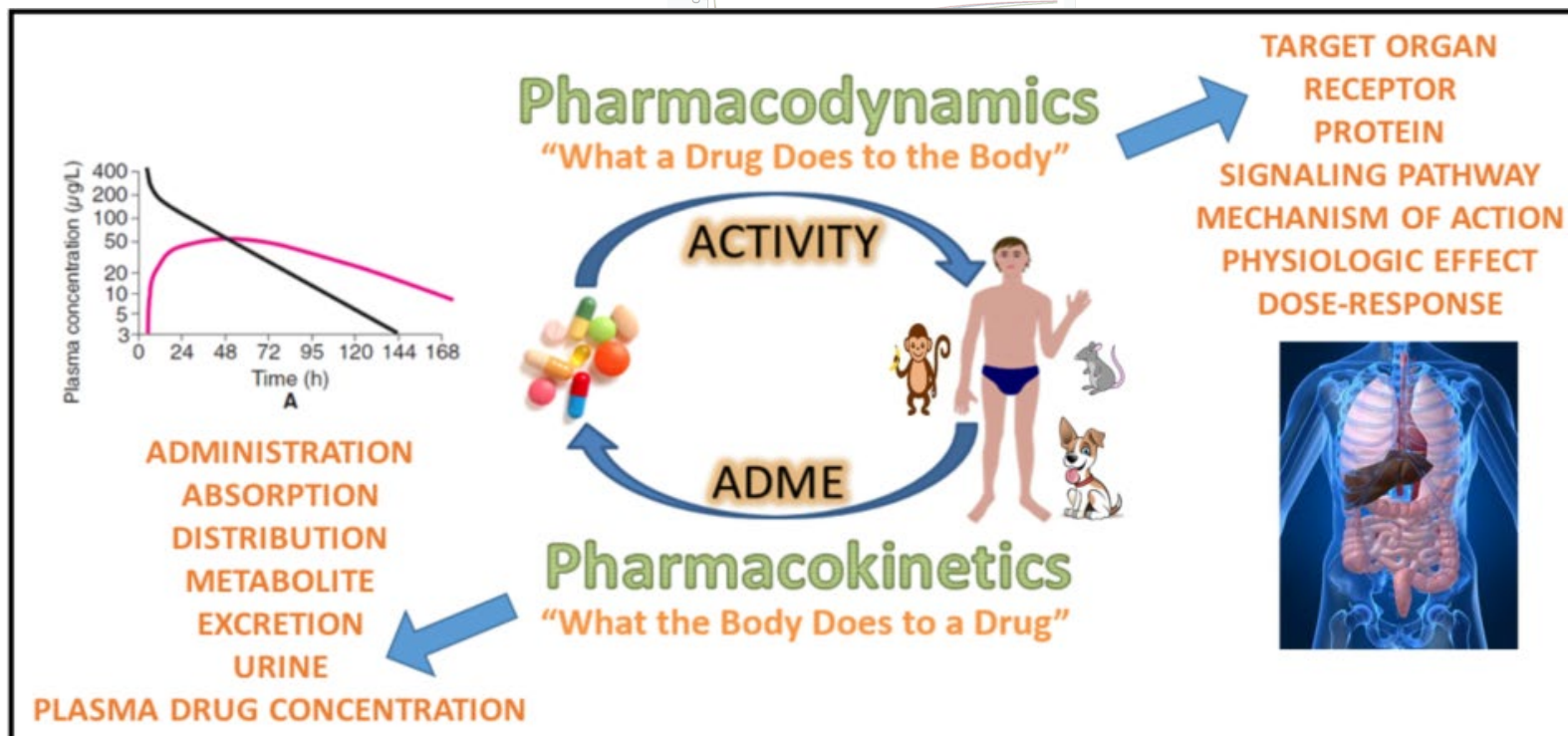
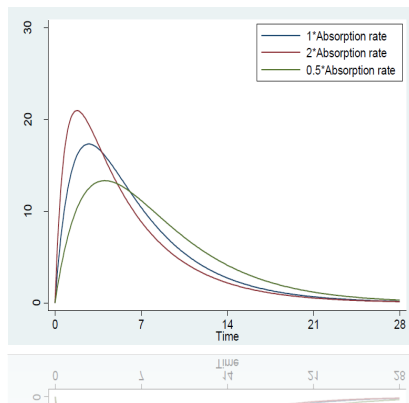
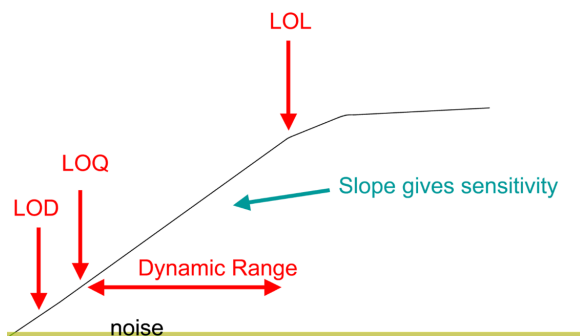


Practical Bioanalytical Method Validation by LC-MS

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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				

$$\text{QC1} \leq 3 \times \text{LLOQ}$$

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Accuracy and precision expressions

True = Actual = Theoretical = Nominal value

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

Accuracy = bias/error/deviation/difference

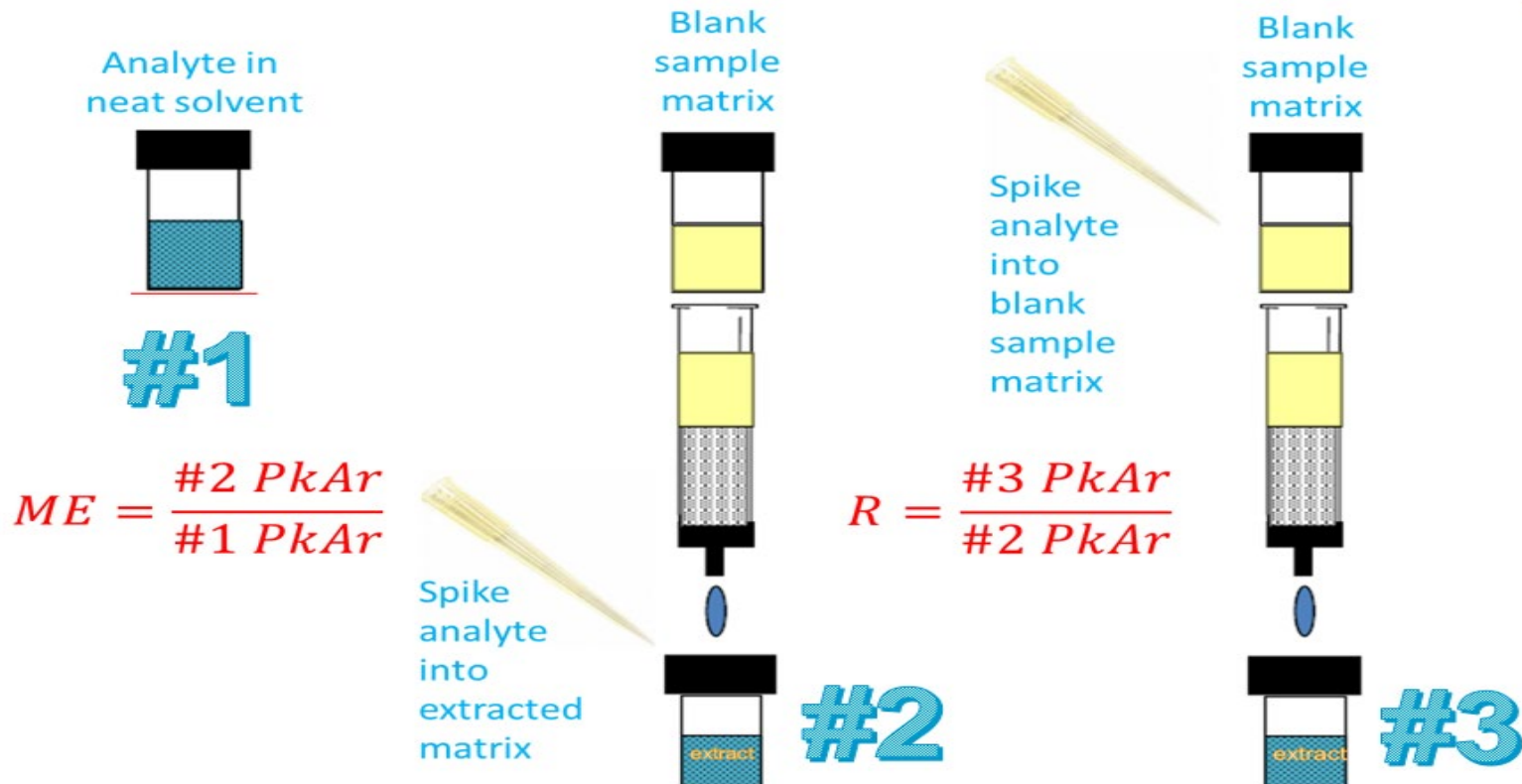
$$\frac{\text{Determined Value} - \text{True Value}}{\text{True value}} \times 100 \quad \pm 15\%$$

Precision: RSD = CV (coefficient of variation)

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Matrix effect and extraction recovery



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

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

	Batch 1	2	3	4	5	6	7	8
A	MP	MP	Blk	Std0	Std1	Std2	Std3	Std4
B	Std5	Std6	Std7	Std8	LQC	MQC	HQC	Sam
C	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam
D	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam
E	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam
F	Sam	Sam	Sam	Sam	Sam	LQC	MQC	HQC

	Batch 2	2	3	4	5	6	7	8
A	MP	MP	Blk	Std0	Std1	Std2	Std3	Std4
B	Std5	Std6	Std7	Std8	LQC	MQC	HQC	Sam
C	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam
D	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam
E	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam
F	Sam	Sam	Sam	Sam	Sam	LQC	MQC	HQC

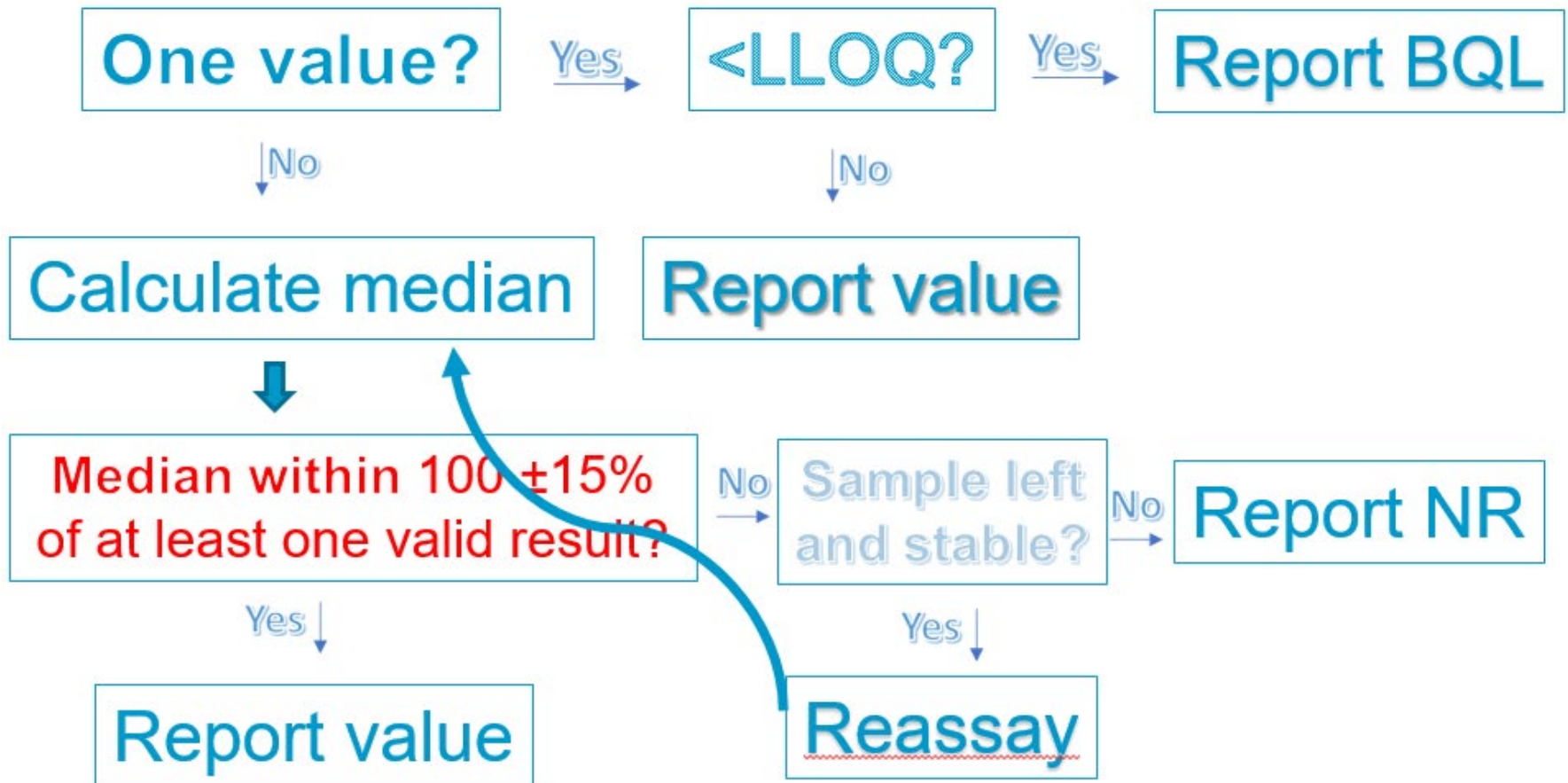
Based on 2018 BMV acceptance
75% **and** a minimum of six standards (6/8 = 75%)
Both batches passed – each batch was
processed **individually**



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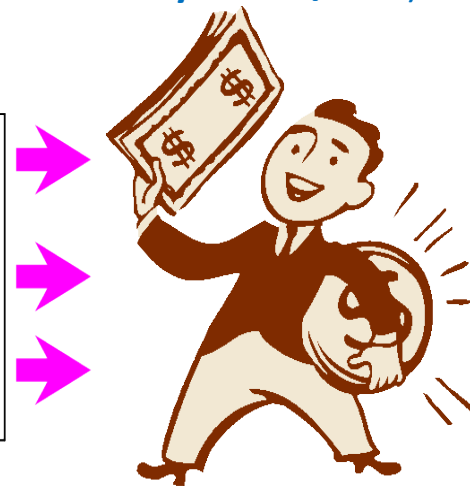
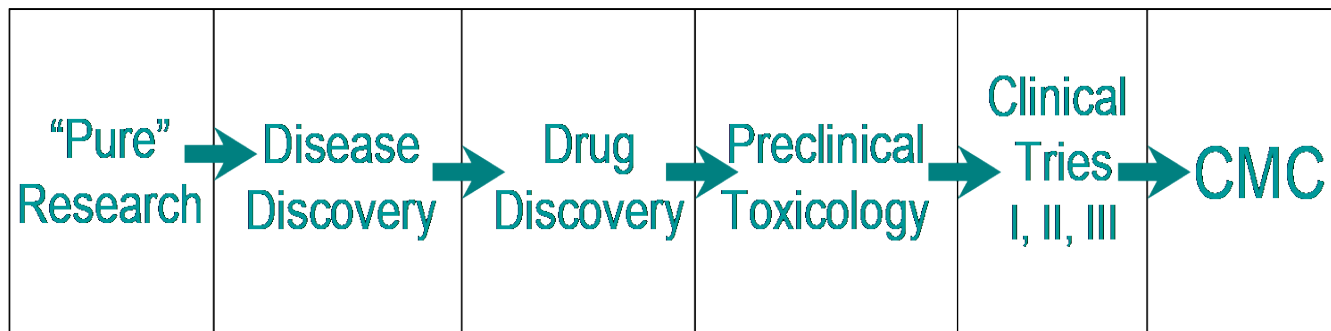
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Decision tree for reporting re-assay results



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GLP GCP GMP

Not regulated



Regulated



Study based

Process based

Good Regulatory Practices (GXP)

Good Regulatory Practices (GXP)