Challenges and Strategies for Bioanalysis following Nanoparticle drug delivery

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Challenges and Strategies for Bioanalysis following Nanoparticle drug delivery

Why nanoparticles → Case study: method Development → Case study: sample analysis

Why nanoparticles?

Why explore nanoparticle formulations?

- Using nanoparticles may influence bio distribution and prolong systemic exposure and so improve delivery of drugs to targets.

- Nanoparticles may be a promising solution to drugs that have not progressed in development due to unfavourable DMPK properties.

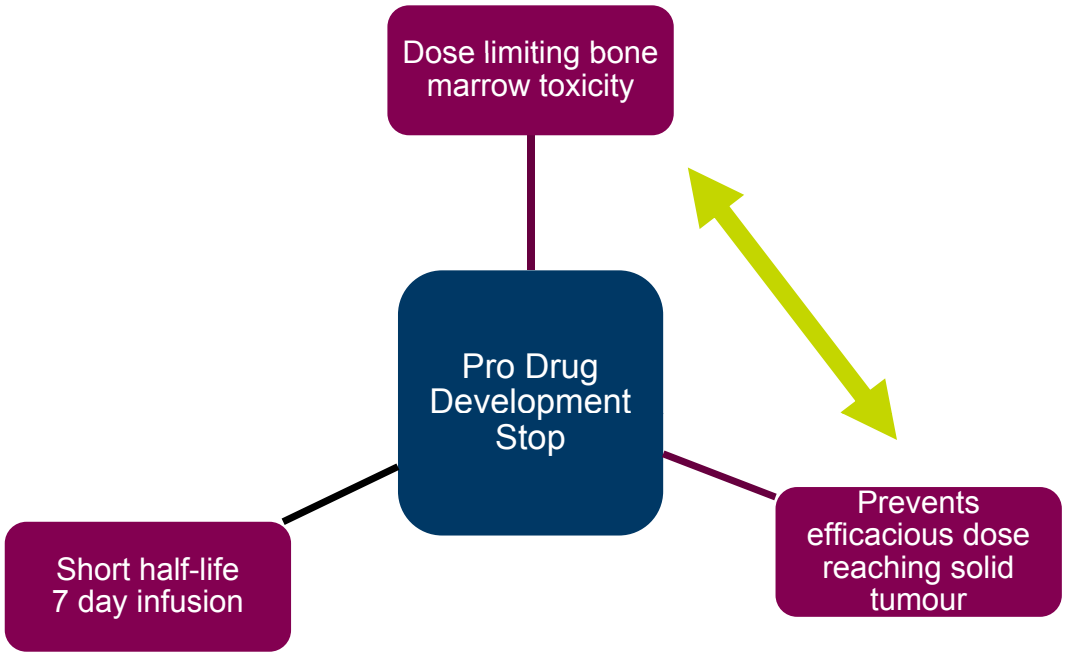
- May be used to improve Therapeutic Index

Case Study – AZD2811 Background

- AZD2811, development molecule in oncology therapeutic area
- Aurora B Kinase plays a pivotal role in regulating the cell cycle, in particular in chromosome segregation
- AZD2811 is a potent selective inhibitor of Aurora B kinase
- Inhibition of Aurora B leads to programmed cell death (apoptosis)

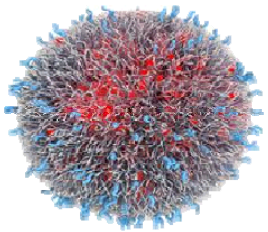
Case Study – AZD2811 Background

- AZD2811 was dosed as a prodrug (AZD1152) and displayed clinical activity, reaching Proof Of Concept in elderly Acute Myeloid Leukaemia (AML) patients, but:



Case study – what if…??

- We could improve delivery of AZD2811 to solid tumours?
- We could use a formulation that would enable dosing of AZD2811 over a short time frame whilst delivering the active drug slowly to the systemic circulation?
  - We may have a drug to treat haematological tumours

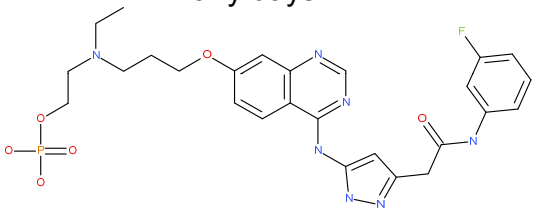


Accurins™ (BIND Therapeutics) nanoparticles

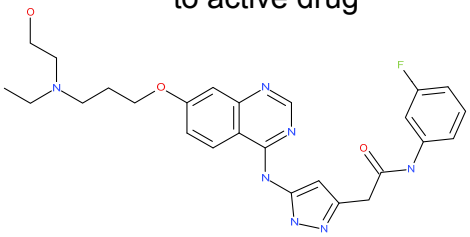
Potential to target tumours with controlled release rate

Case study – concept

1) Prodrug infused over many days

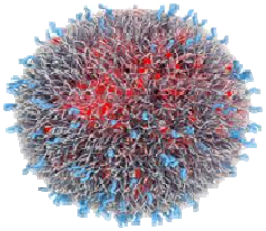


2) Prodrug rapidly converted to active drug

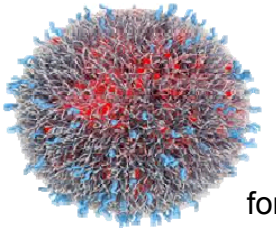


3) Drug eliminated

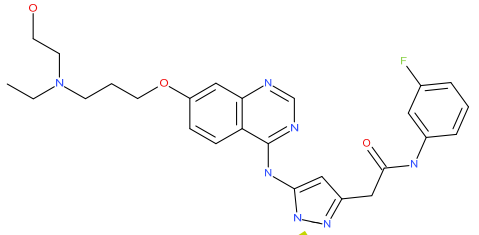
Conventional approach – active drug concentration balance of drug infusion and drug elimination rate



1) Prodrug infused over ≤ 2 hours



2) Nanoparticle circulates for > 1 day releasing active drug



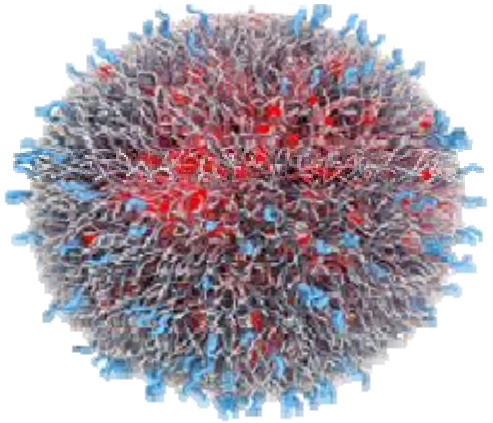
3) Nanoparticle eliminated

4) Drug eliminated

Nanoparticle approach – active drug concentration balance of drug release, drug elimination and nanoparticle elimination rate

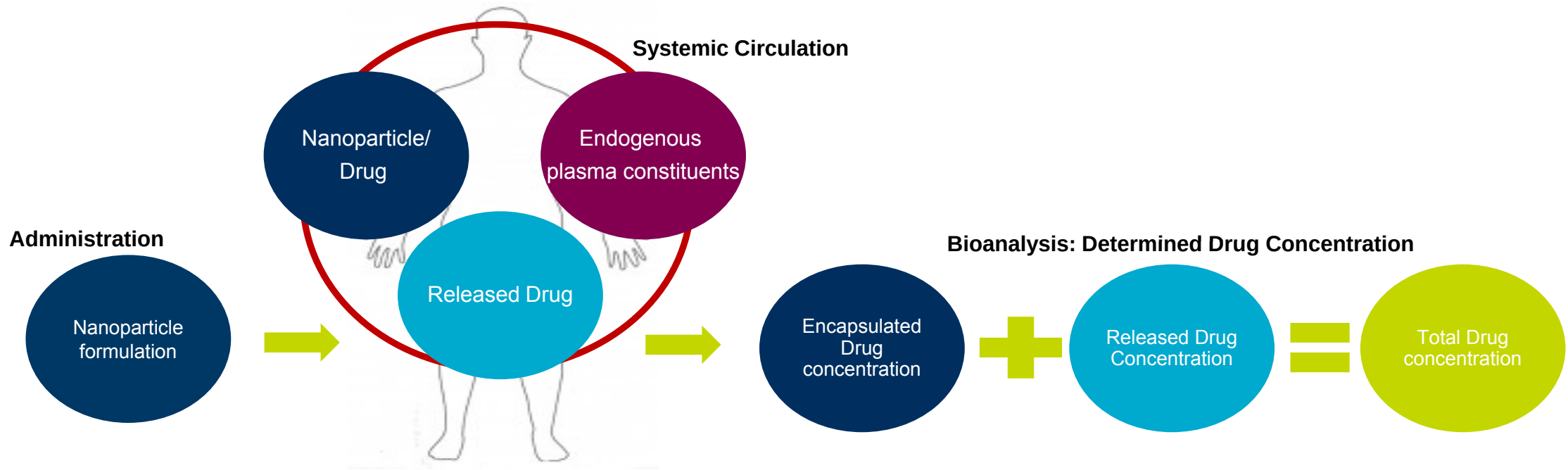
Case study – method development

Accurin™ Nanoparticle for AZD2811



- Particles are a polymer mix with a core containing active drug.
- Particles have a stealth coating of PEG to prevent rapid removal by macrophages
- Have a size of about 100nm

Accurin™ Nanoparticles - Bioanalysis



Nanoparticle formulation: The drug is encapsulated in the nanoparticle

Encapsulated drug: The nanoparticle/drug in the biological matrix

Release drug: The drug no longer associated with the nanoparticle

Total Drug: Released and encapsulated drug in the biological matrix

Pre-clinical Investigative Study Support

Study Endpoint: Safety

- **Total analysis only (encapsulated + released drug)**

Study Endpoint: PK/PD

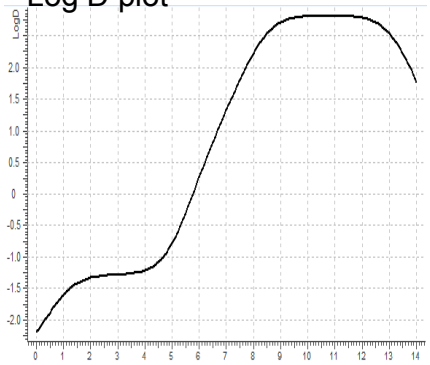
- **Released Drug analysis**
  - **(Total – Encapsulated) = released** ✗
  - **Released drug measurement** ✓
  - **Surrogate (metabolite) measurement** ✓

Bioanalysis: Total AZD2811 and Acid Metabolite

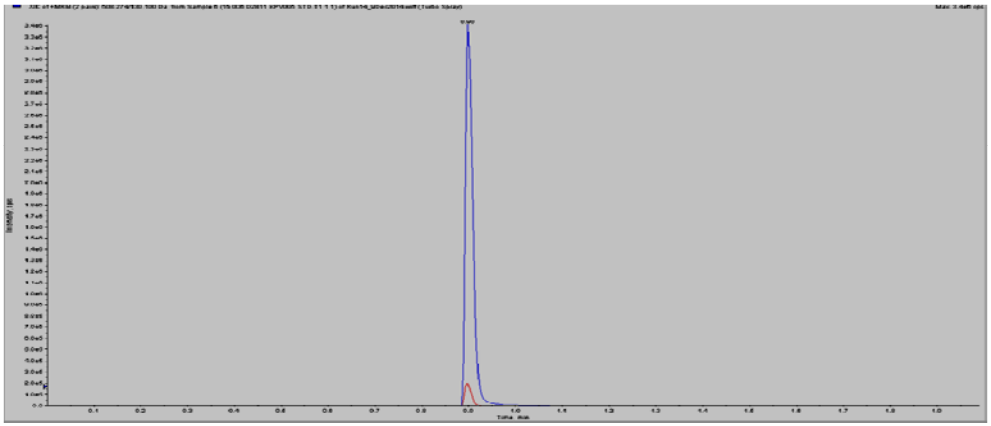
Protein precipitation, dilute extracts analysis by UPLC-MS/MS

AZD2811

Log D plot

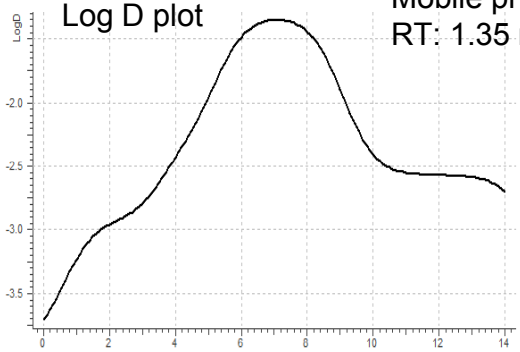


Column: Acquity UPLC BEH C18 1.7 µm
Mobile phase: acidic:
RT: 0.9 mins

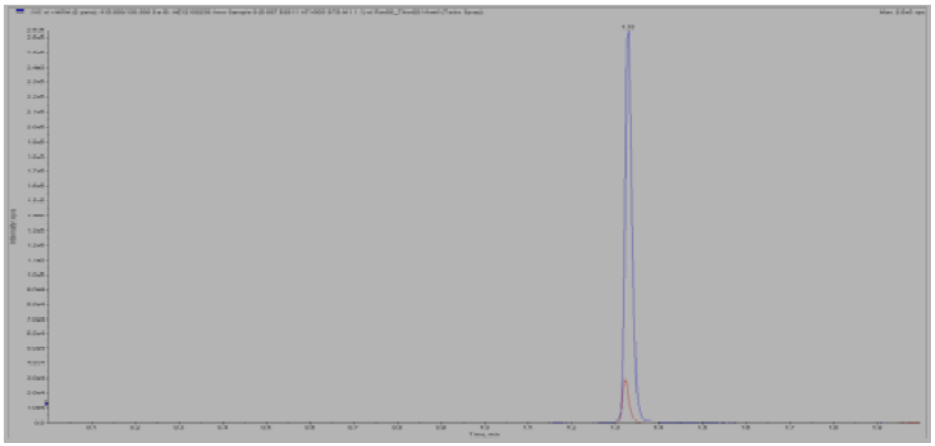


Metabolite

Log D plot



Column: Acquity UPLC BEH C18 1.7 µm
Mobile phase: basic
RT: 1.35 mins



Bioanalysis: Released AZD2811 - Challenges

What acceptance criteria do we use?

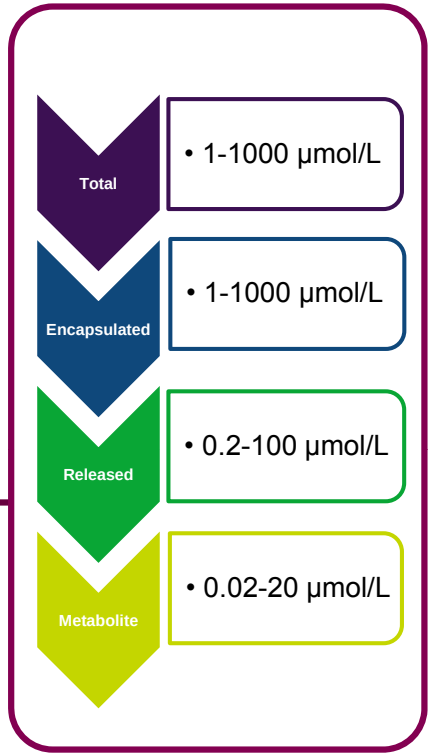
Nanoparticle Stability

Nanoparticles designed to 'leak'

Sample Handling keep chilled to minimise diffusion

Extraction Stability avoid acid, base and organic solvents to prevent bursting

Assay Dynamic Ranges



Compound & Formulation

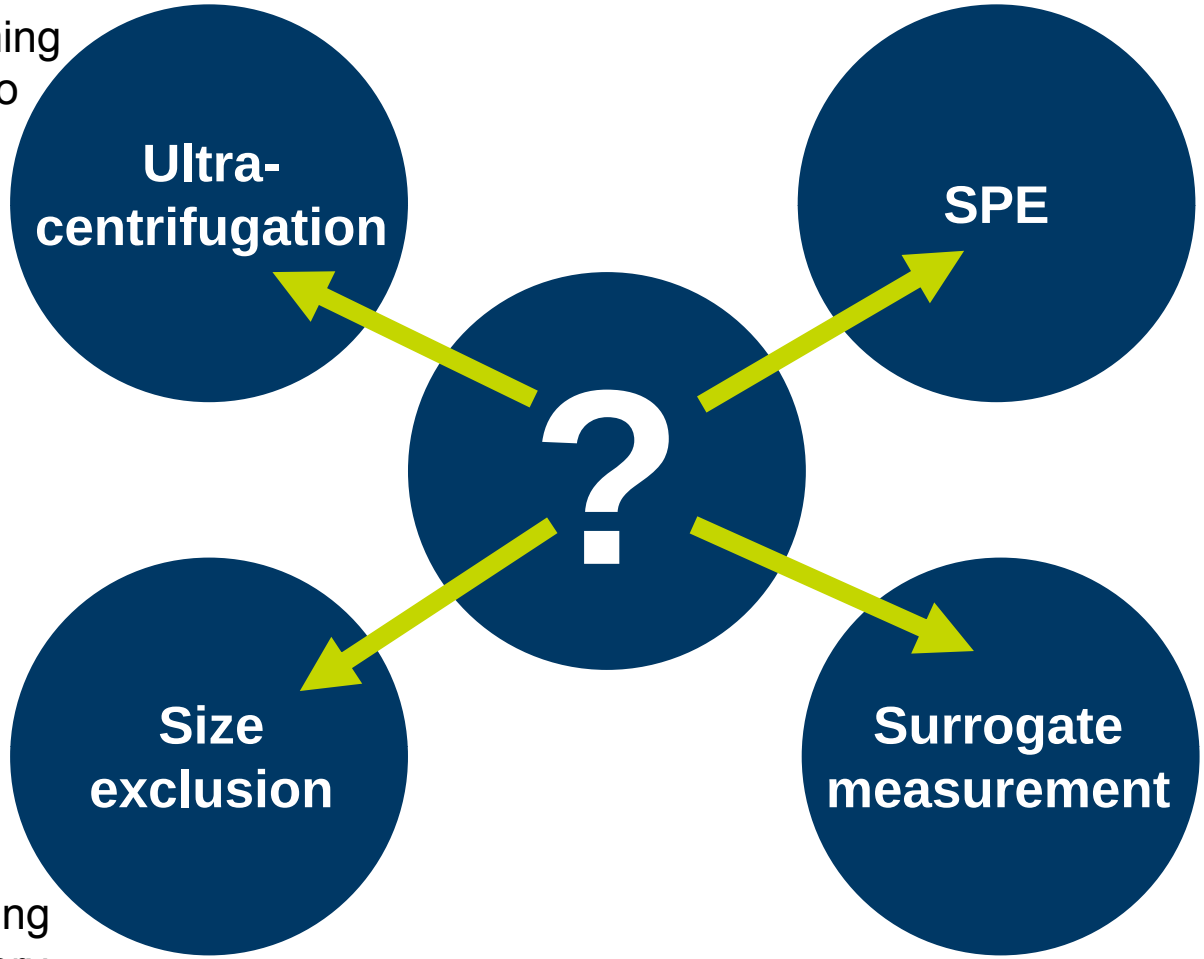
Parent has metabolite as impurity

Released parent in nanoparticle formulation

How do we separate fractions?

Approaches to measuring released AZD2811

•Time consuming
•Which layer to sample?

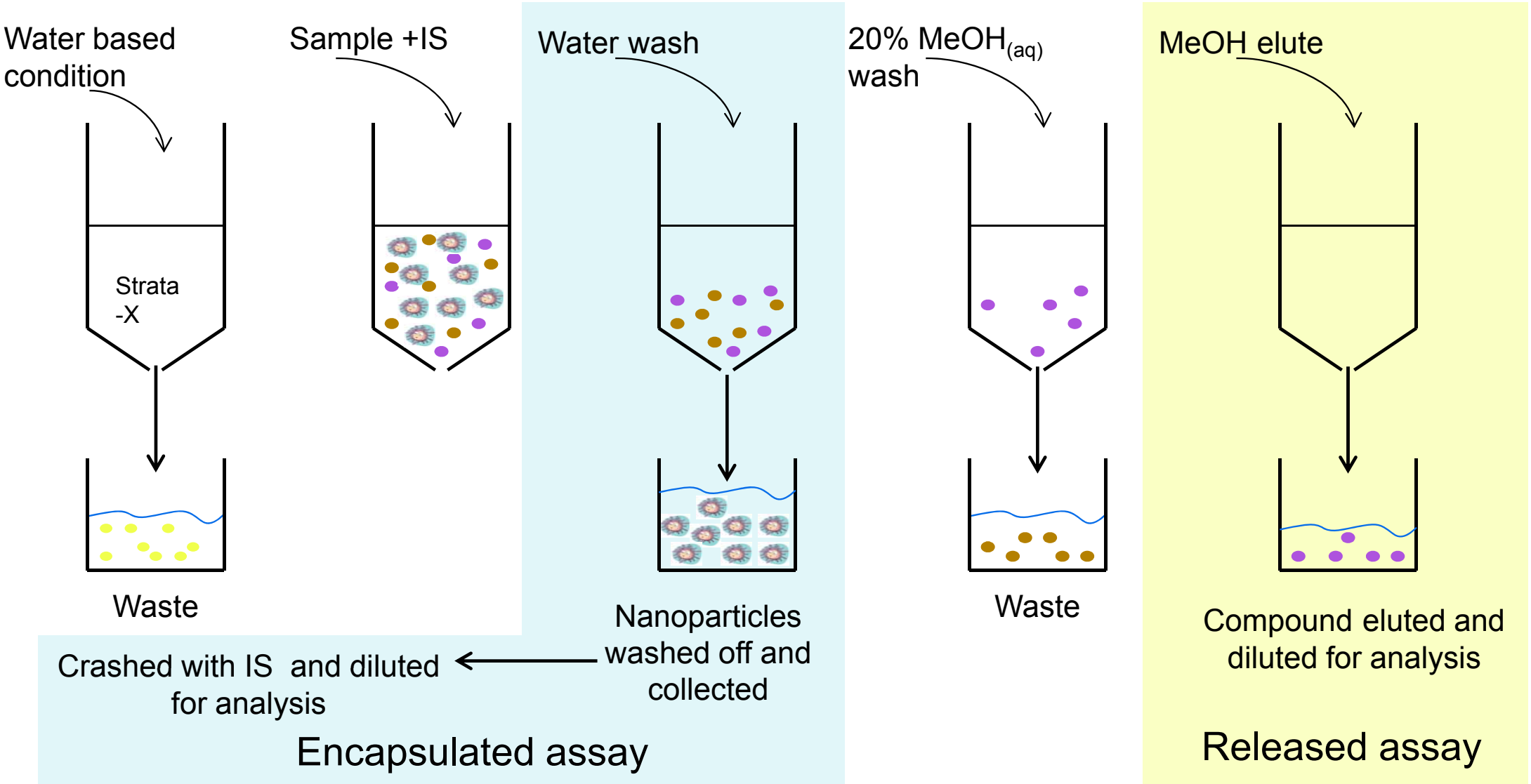


• Easy to perform/automate
•Sample throughput good
•When to add IS?

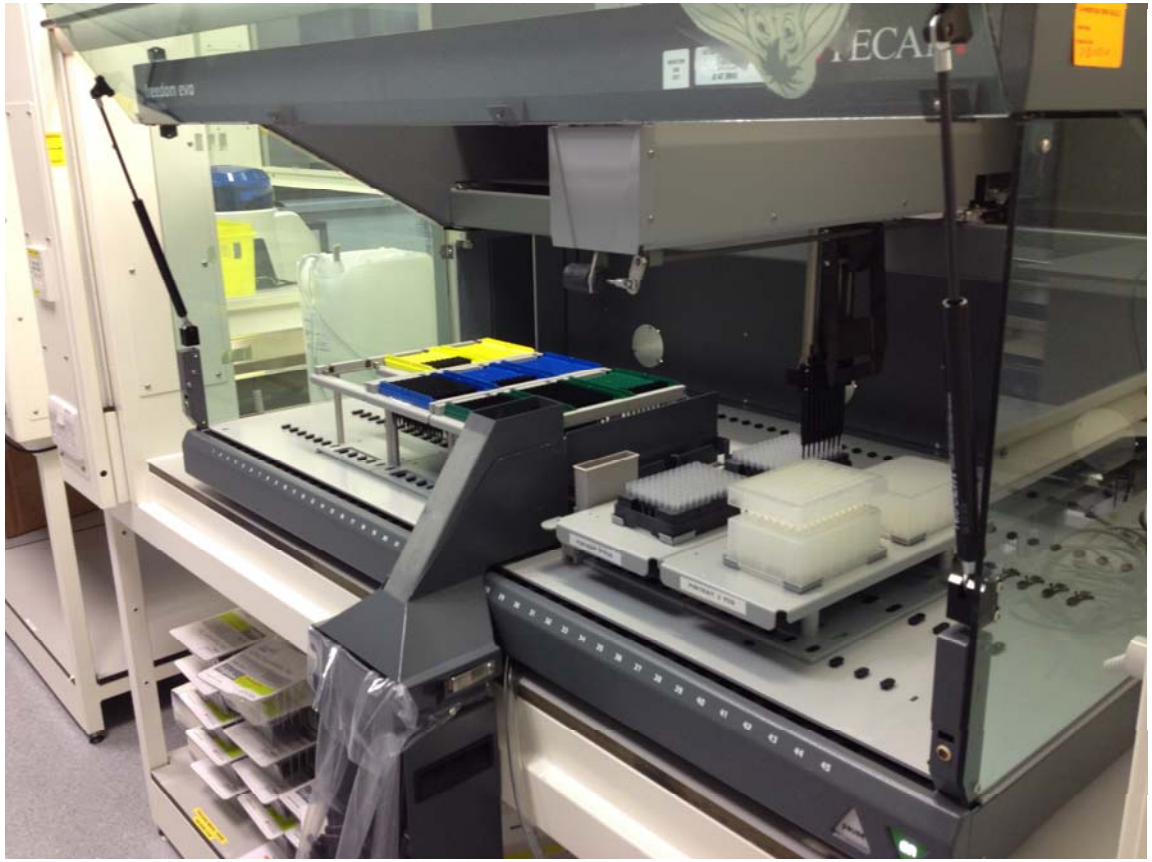
•Time consuming
•Drug interaction with phase resulting in reduced recovery

•Preclinical & clinical data available
•Standard bioanalytical methodology

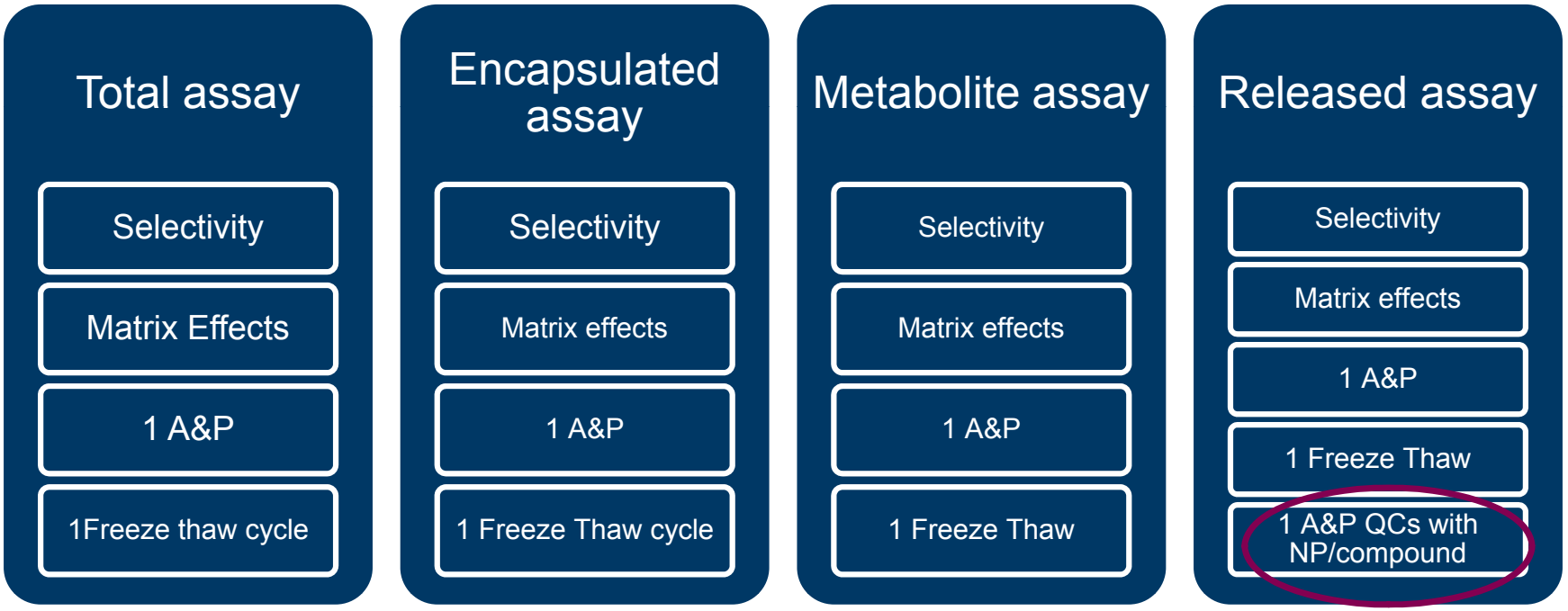
Encapsulated and released solid phase extraction



A major team contributor….



Tiered approach to assay qualification

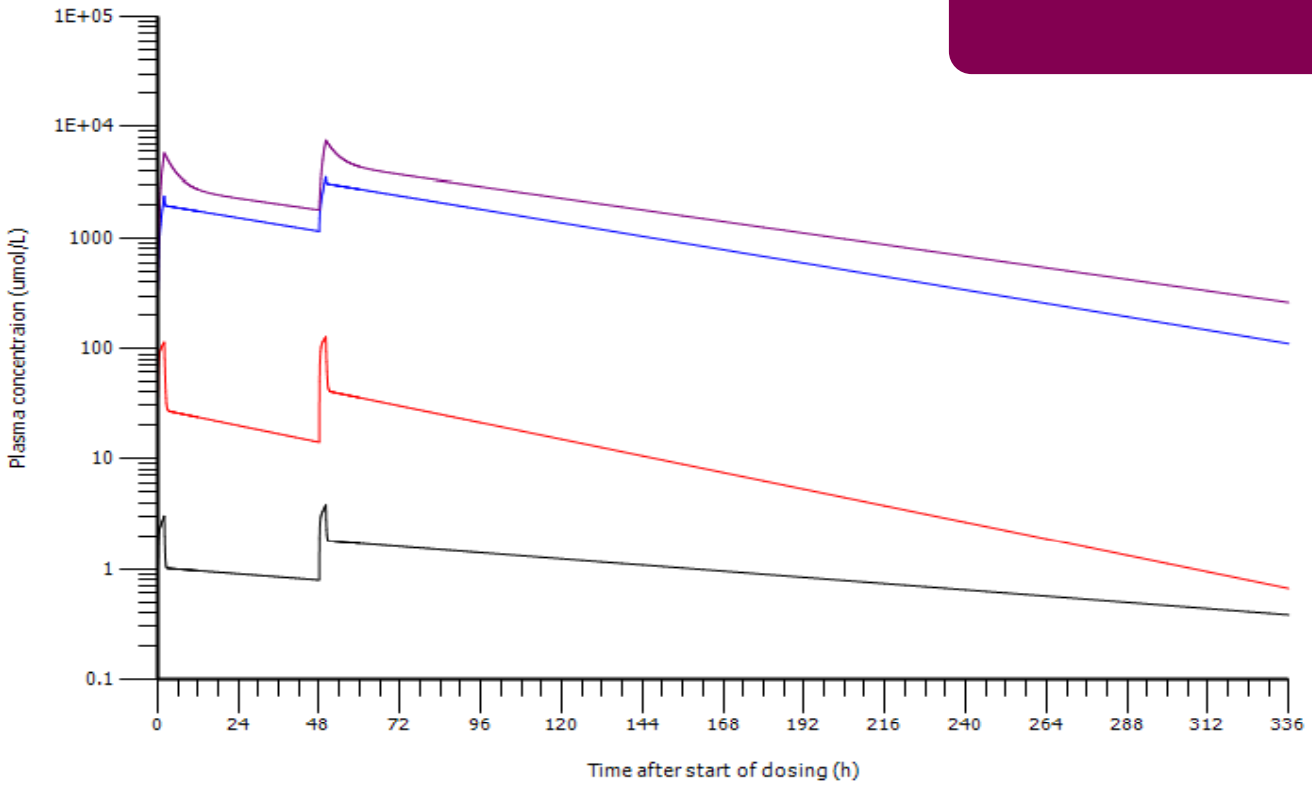


Acceptance Criteria:
Bias/CV 20% (25% at LLOQ)

Case study – sample analysis

Example: Profile simulations

Cyclic administration-
Day 1 & Day 3: 30min infusion

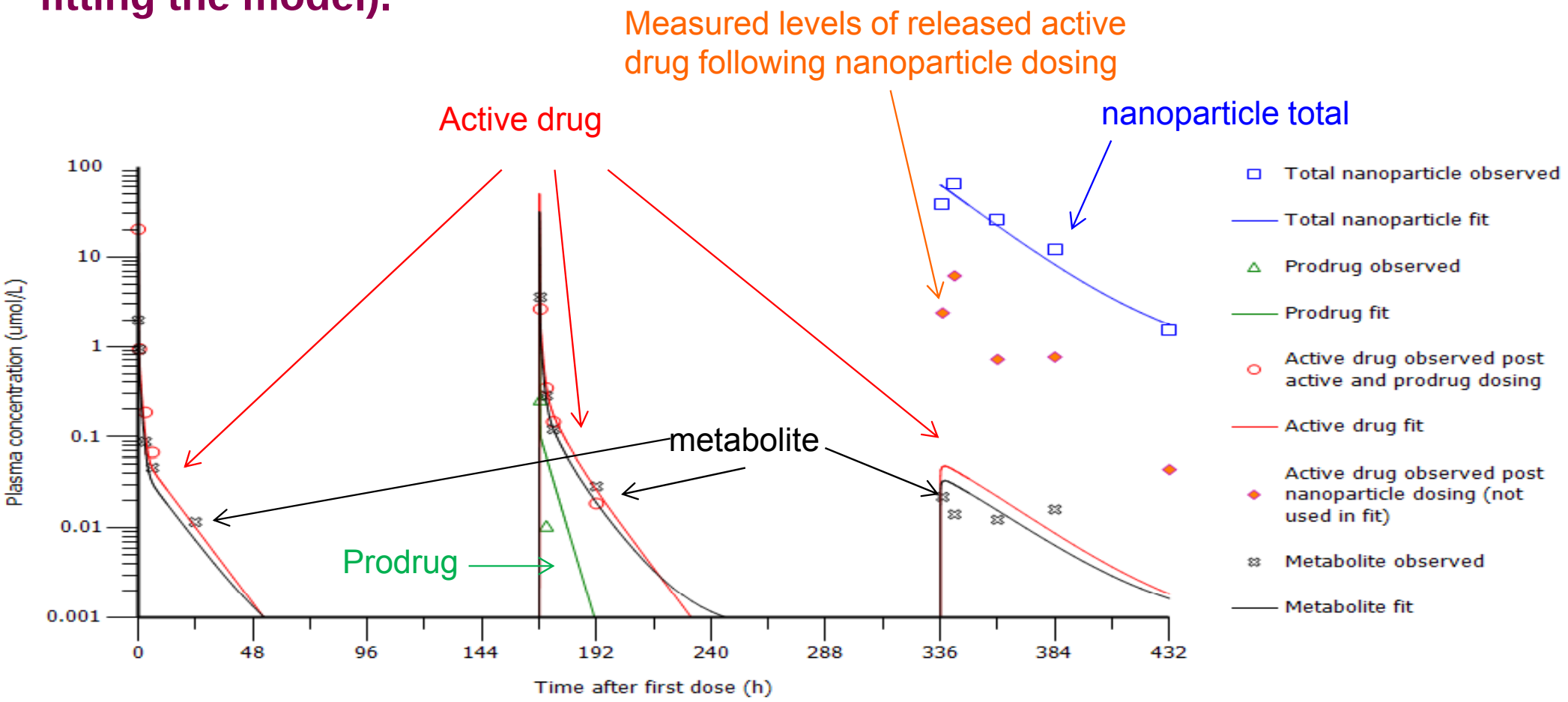


Encapsulated & Total

Released

metabolite

Example plot: *an individual rat* dosed with active drug (9.6 umol/kg) at 0 h, prodrug (22 umol/kg) at 168h and nanoparticle (2.3 umol/kg) at 336 h with the measured nanoparticle active levels overlaid (note: these are not used in fitting the model).



Released assay - control of nanoparticle burst

During extraction are we bursting any nanoparticles?

Nanoparticle conc (umol/L)	3		50		800	
Released Conc (umol/L)	0.3		5		80	
	Conc.	Bias (%)	Conc.	Bias (%)	Conc.	Bias (%)
Run01	0.378	26	5.20	4	99.6	25
	0.365	22	6.07	21	104	30
Run02	0.363	21	6.68	34	100	25
	0.288	-4	6.63	33	94.4	18
Run3_	0.299	-0.3	4.74	-5	107	34
	0.324	8	4.80	-4	94.0	18
Mean	0.336	NC	5.69	NC	99.8	NC
CV (%)	11.3	NC	15.6	NC	5.15	NC
Bias (%)	1.2	NC	-1.4	NC	2.5	NC
n	6	6	6	6	6	6

QC's contain 'released' AZD2811 & nanoparticle formulation

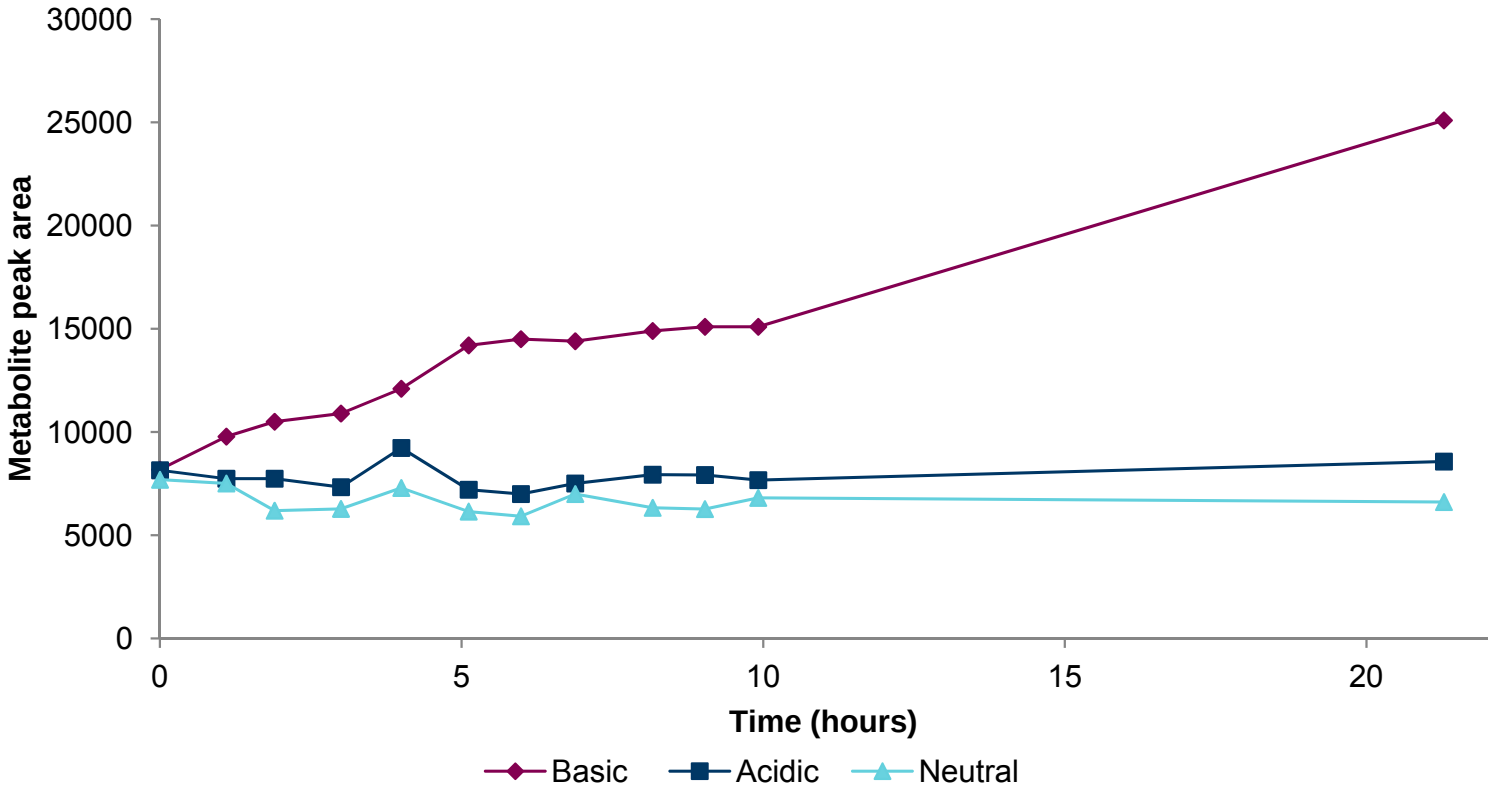
Measured 'released' AZD2811 concentrations

Mainly positive bias observed accounted for by 2% AZD2811 release rate in nanoparticle formulation.

Therefore: In Control of assay

For GLP studies metabolite surrogate method chosen

Stability of AZD2811 & Impact on Metabolite Quantification: Stability of AZD2811 (2 µmol/L) in reconstitution solvents

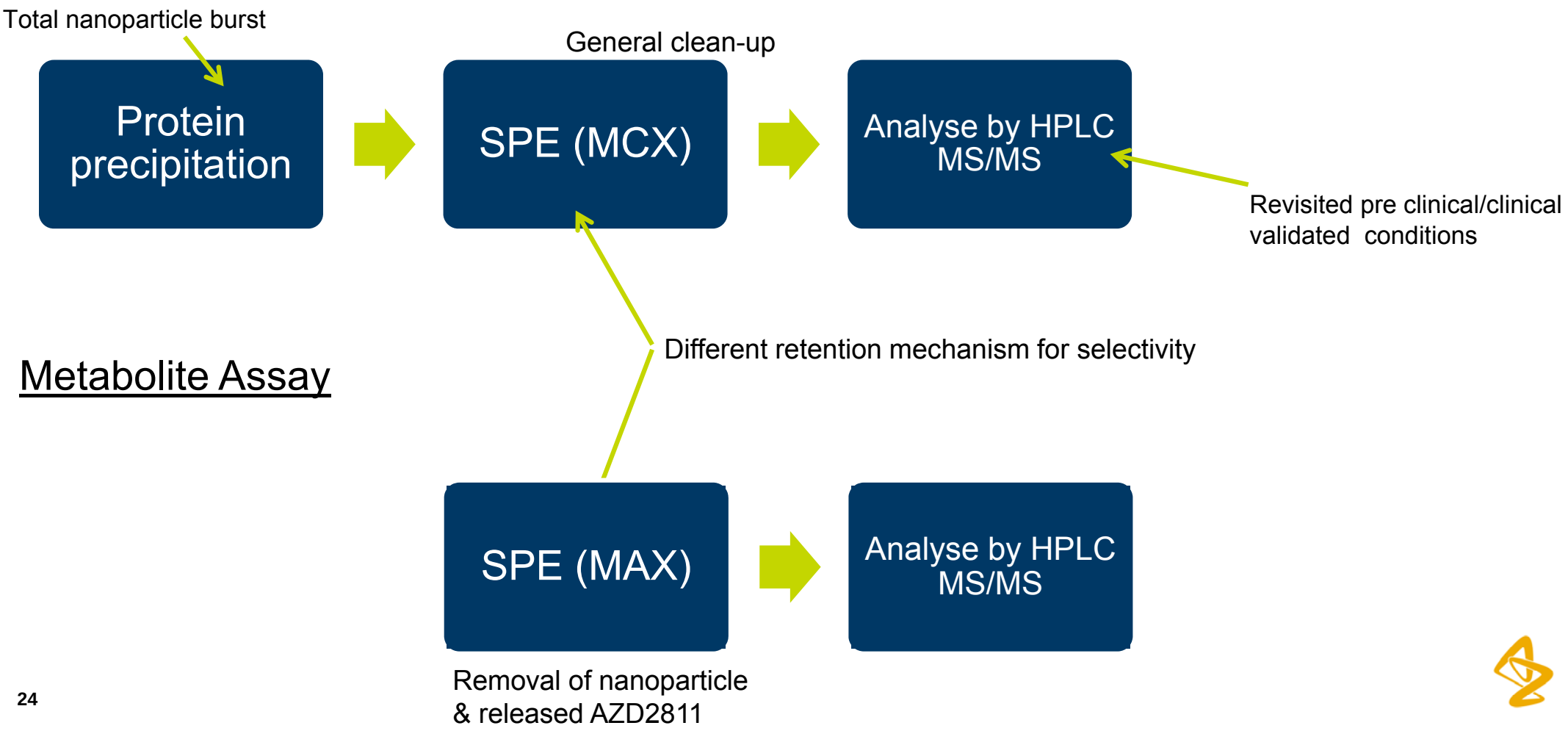


Stabilised plasma samples with acid and controlled pH in reconstitution solvent

Nanoparticle burst but using total analysis for AZD2811

GLP Assays-Total and Metabolite

Total AZD2811 Assay



Additional Validation considerations:

Selectivity	• With blank nanoparticles • With haemolysed blood
Matrix effects	• With blank nanoparticles • With haemolysed blood
Recovery	• With blank nanoparticles • With haemolysed blood
Accuracy & Precision	• Using Nanoparticle formulation QCs
Stability	• Using Nanoparticle formulation QCs • Stressed AZD2811 QCs

Metabolite ISR

Animal number	Dose mg/kg	Time of sampling(h)	Original conc. (nmol/L)	Original batch ID	Reassay conc. (nmol/L)	Reassay batch ID	% Bias
3101M	10	Day 1 35mins	104	PB01	69.0	PB18	-40.2[a]
3101M	10	Day 1 48h	71.0	PB01	68.3	PB18	-3.9
3103M	10	Day 1 35m	93.6	PB01	80.9	PB18	-14.6
3103M	10	Day 1 48h	80.6	PB01	71.8	PB18	-11.4
5101M	30	Day 1 35m	213	PB01	163	PB18	-26.7[a]
5101M	30	Day 1 48h	148	PB01	160	PB18	7.8
5602F	30	Day 1 35m	190	PB02	181	PB18	-4.6
5602F	30	Day 1 48h	113	PB02	177	PB18	43.7[a]
3102M	10	Day 29 35m	120	PB02	127	PB18	5.2
3102M	10	Day 29 48h	66.6	PB02	104	PB18	43.4[a]
3603F	10	Day 29 35m	81.2	PB02	76.9	PB18	-5.4
3603F	10	Day 29 48h	65.3	PB02	100	PB18	42.3[a]
5102M	30	Day 29 35m	217	PB03	261	PB18	18.3
5102M	30	Day 29 48h	140	PB03	197	PB18	34.2[a]
5603F	30	Day 29 35m	226	PB03	351	PB18	43.3[a]
5603F	30	Day 29 48h	118	PB03	173	PB18	38.4[a]
3102M	10	Day 31 24h	116	PB03	133	PB18	14.1
3102M	10	Day 31 120h	76.4	PB03	74.6	PB18	-2.3
3103M	10	Day 31 35m	124	PB03	147	PB18	16.6
3103M	10	Day 31 120h	63.2	PB03	69.4	PB18	9.3
5103M	30	Day 31 35m	427	PB04	372	PB18	-13.8
5103M	30	Day 31 120h	168	PB04	195	PB18	15.1
5601F	30	Day 31 35m	365	PB04	305	PB18	-18.0
5601F	30	Day 31 120h	82.6	PB04	118	PB18	35.5[a]

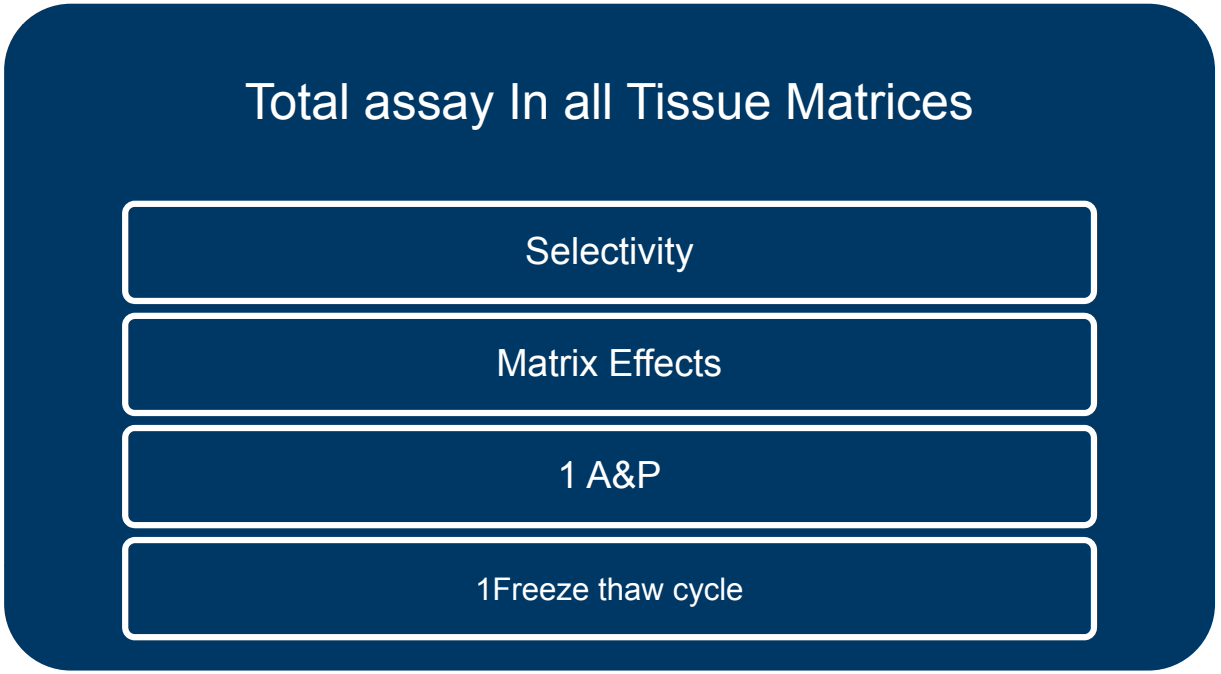
Failed acceptance

ISR Investigations show

- No obvious trend with Day or collection times.
- AZD2811 was present in the extracts. MAX extraction is not 100% selective for metabolite.
- Reinjection of study samples show an increase in the metabolite concentrations.
- The AZD2811 appears to be converting to metabolite in the extracts. The impact will be dependent on the amount of AZD2811 present in the extracts, dependent on the concentration of the metabolite present and the injection order/time of preparation of the extract.

Biodistribution studies -tiered approach

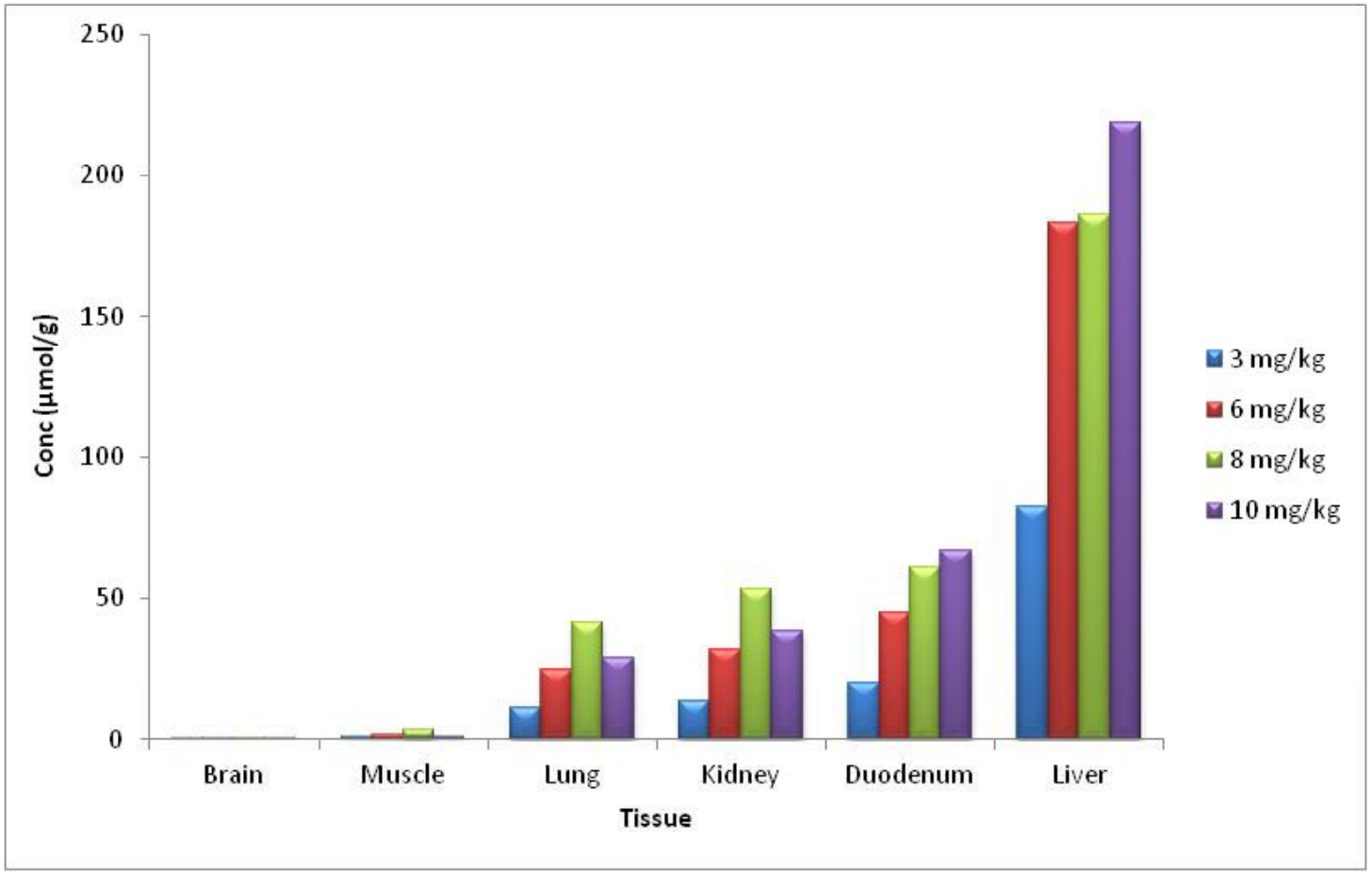
Due to the change of formulation the bio distribution of AZD2811 with nanoparticle delivery was assessed.



Acceptance Criteria:
Bias/CV % 20% (25% at LLOQ)

Bio distribution

Distribution of Total AZD2811 in NHP tissues following repeated IV infusion in a nanoparticle



Nanoparticles characteristically show tissue distribution dictated by uptake by the reticuloendothelial system

Summary

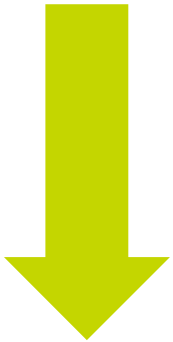
- Understanding the nanoparticle formulation and the analyte properties is key for method development.

- Accurin nanoparticles did not appear to impact the bioanalytical methodologies developed.
- The total AZD2811 analysis has been used for the safety exposure evaluation

- The main challenge has been measuring the released AZD2811 concentration for exposure vs PD evaluation

Into the Clinic

Total analysis used for the safety margins
For PD would like to understand released concentrations



Assay learning/understanding has been key in the transfer to CRO for clinical analysis
Alternative techniques for released measurements to be evaluated

Acknowledgements

- Jennifer Ayres
- Joanne Wilson
- Aaron Smith
- Mike Walker
- Colin Howes
- Bind Therapeutics