The use of Dried Plasma Spots (DPS) and Dried Urine Spots (DUS) for LC/MS/MS assays

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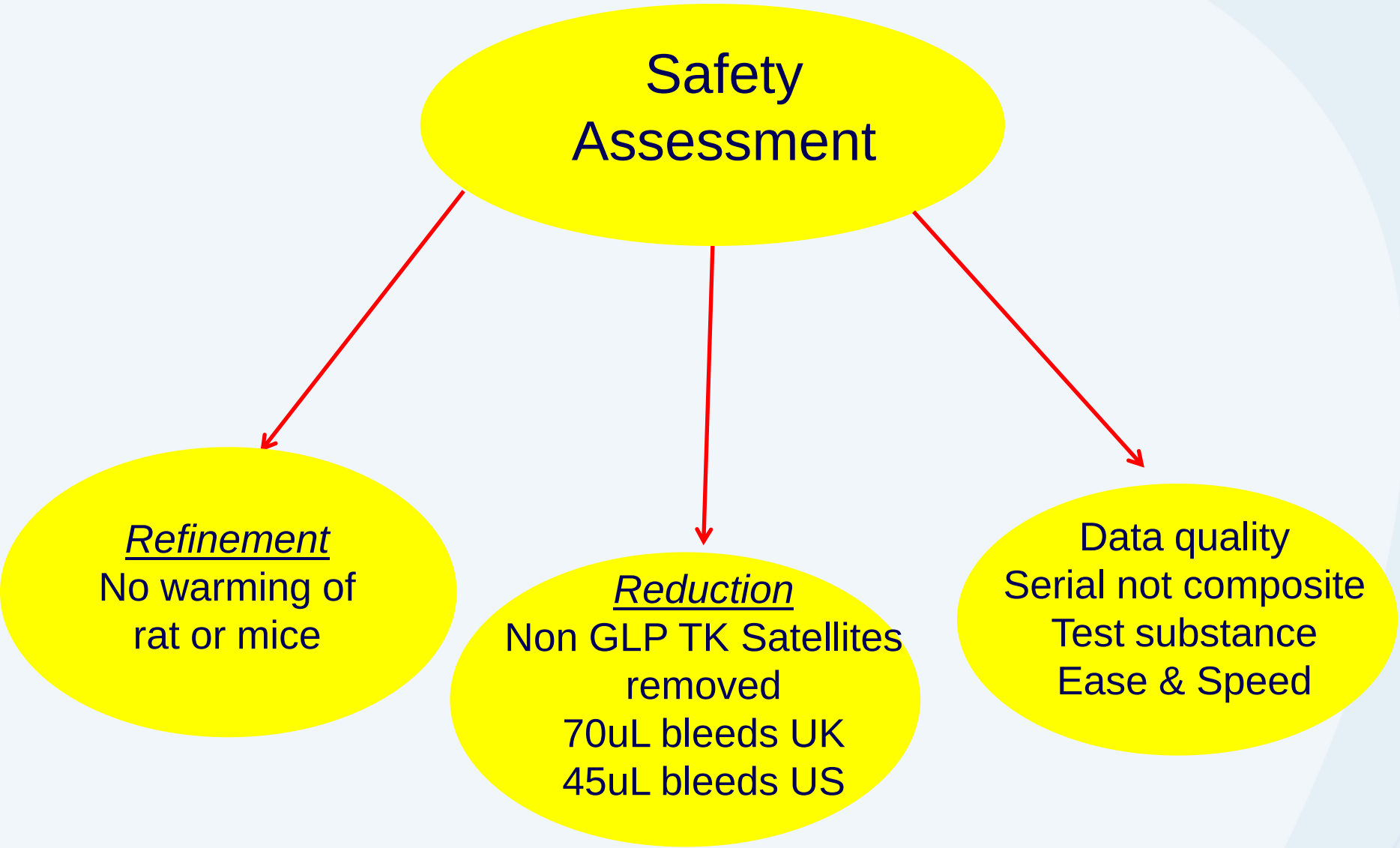
Status of DBS Sampling at GSK*

- 231 DBS bioanalytical methods validated for 105 compounds
- Studies **Completed**

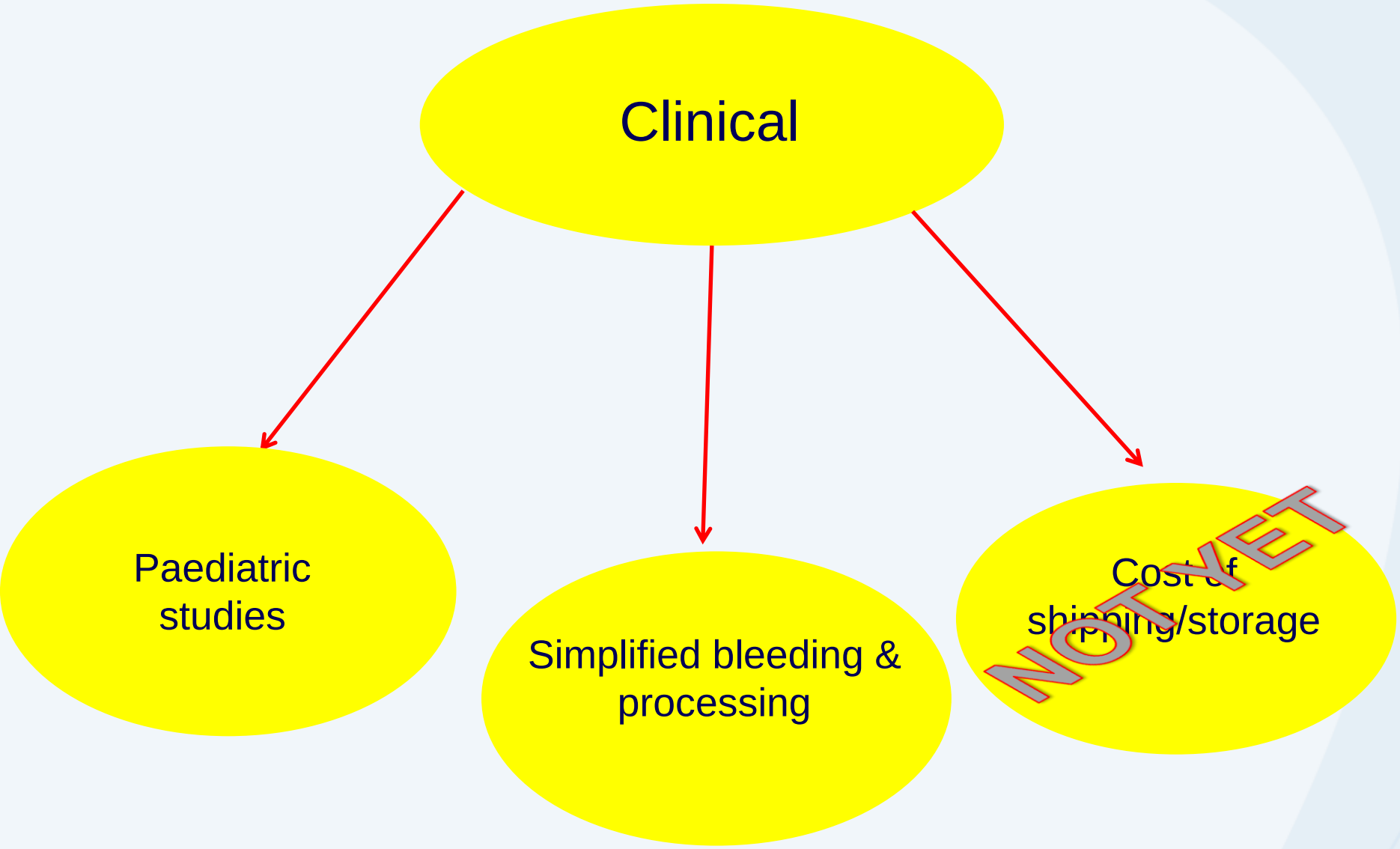
	Studies	Analytes	Samples
Pre-Clinical nonGLP	168	85	20,357
Pre-Clinical GLP	86	35	18,829
Clinical	9	15	4,027

* as of 20 May 2010 (does not include incurred sample reanalysis)

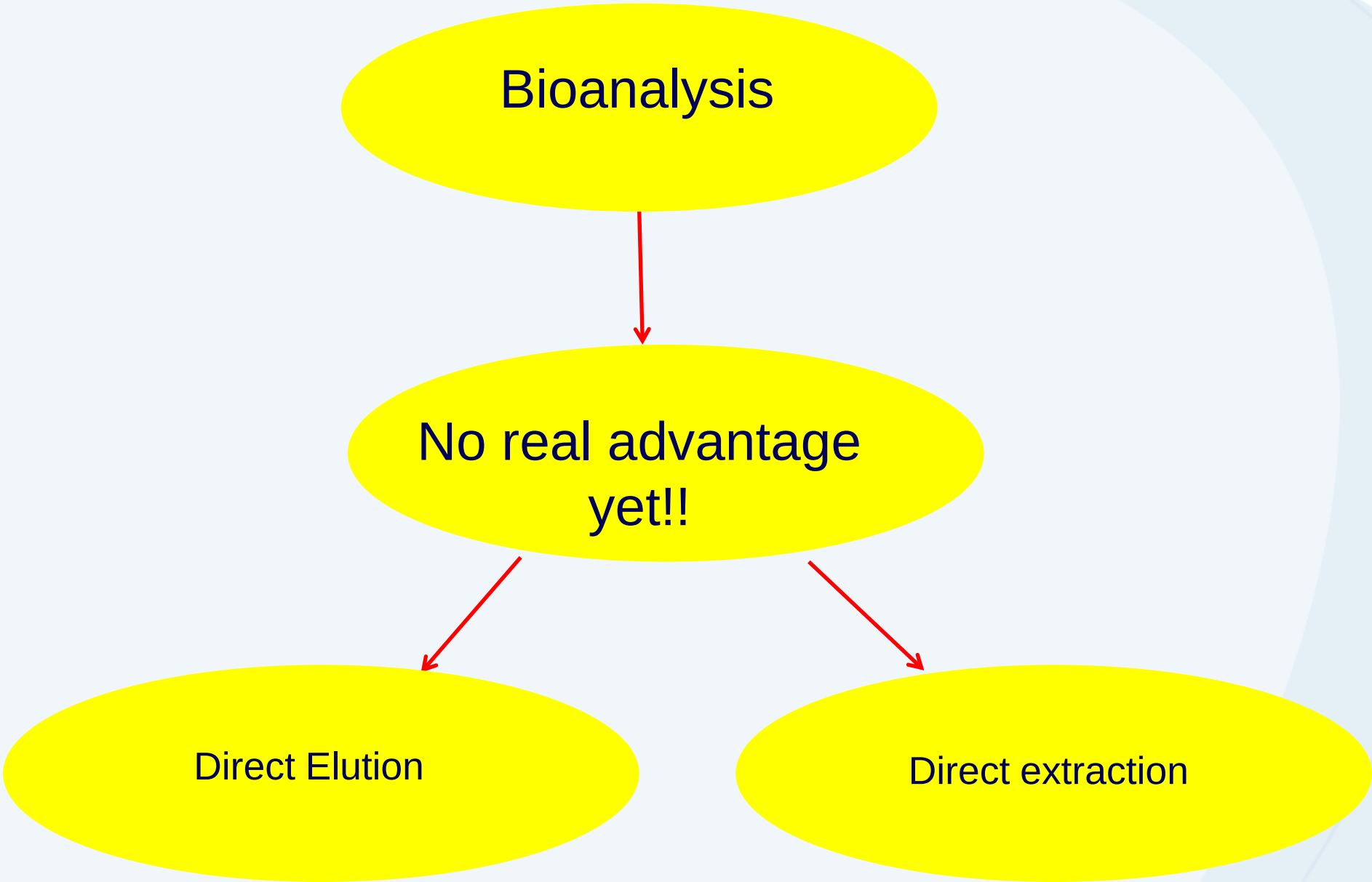
Have the benefits of DBS been realised at GSK



Have the benefits been realised - cont.



Have the benefits been realised - cont.



What can we do to help Clinical?

- Cost savings for clinical will come after phase 1
  -Shipping and storage

GSK estimate if all TK and Clinical samples utilised card technologies we could save between 5 and 8 million pounds a year

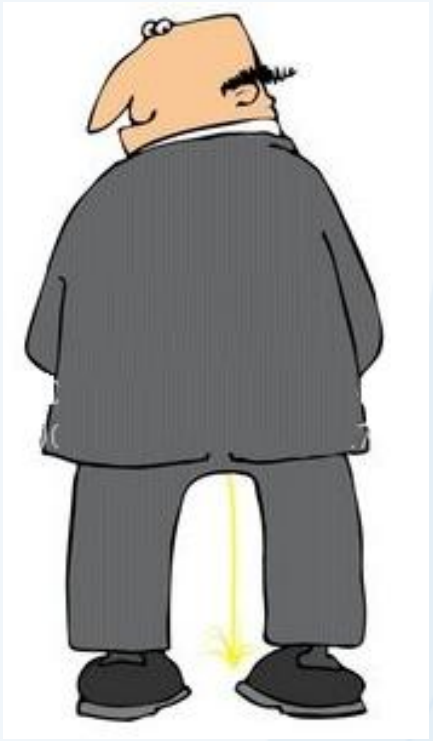
- We can't change easily from plasma to blood

- Why not use Dried Plasma Spots?
- Why not use Dried Urine Spots?

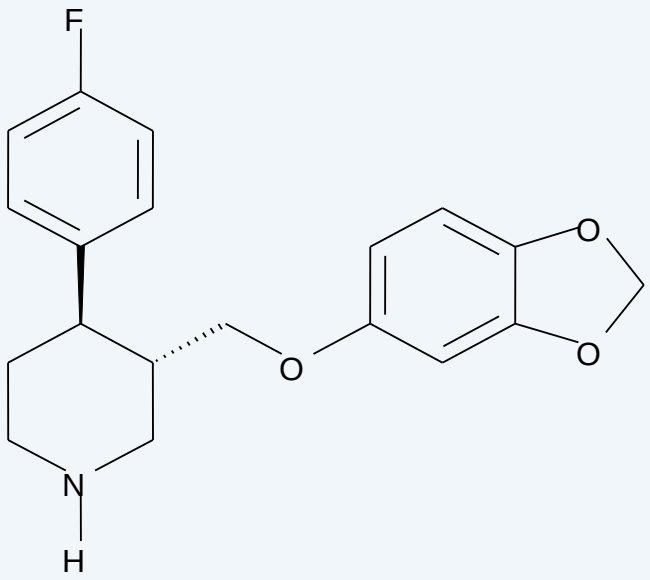
DPS advantages

- DPS
  - Cost savings
  - Ease
  - Utilize new technologies
    - Direct elusion/ionisation

- DUS
  - Cost savings
  - Scientific
    - Solubility
    - Homogeneity
  - Ease
  - Utilize new technologies
    - Direct elusion/ionisation
  - Its much nicer



Full DPS validation for Paroxetine in human plasma



Chiral

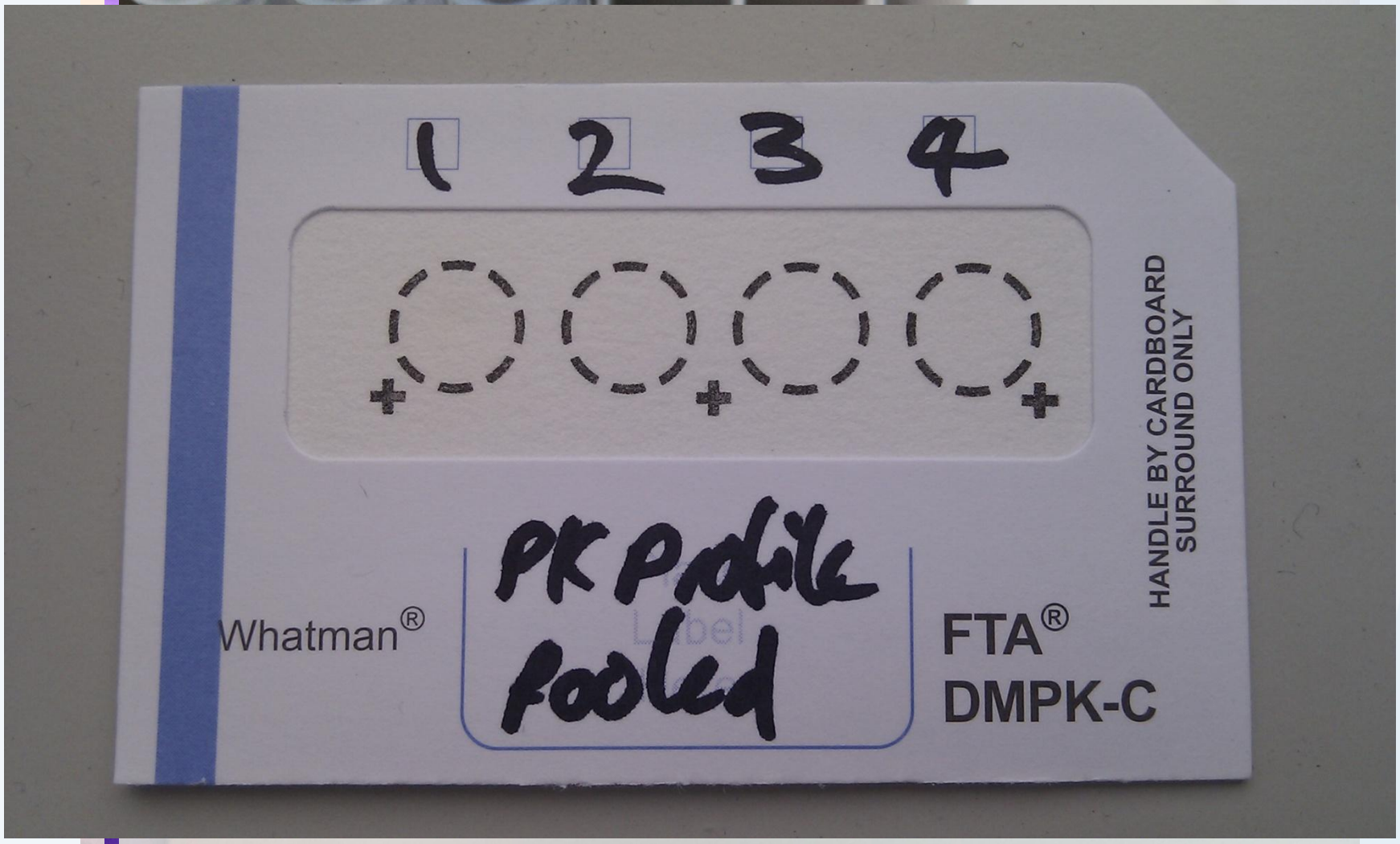
- 20uL spots
- 6mm punch
- 100ul I.S. extract (70:30 MeOH/H_20)
- Shake and decant supernatant
- Inject on reverse phase chromatography
- LC/MS/MS API-5000

- Assay range
  - 0.2-200ng/mL

Validation contents

- Linearity
  - Duplicate calibration lines (front & back)
- Precision, accuracy, sensitivity & reproducibility
  - 5 concentrations, 6 replicates
  - 3 occasions for 1st pre-clin species & human
  - Single occasion for subsequent species
- On card stability
  - 2 concentrations (VC2 & 4), 6 replicates desiccated at room temp for 35 days
- Processed sample stability
  - Re-inject validation QCs with fresh calibrants after storage at room temp for 24 hrs
- Selectivity
  - Total blanks & blanks from 6 different sources
- Assay robustness to pipetting error (10 - 20µL)
  - 2 concentrations (VC2 & 4), 6 replicates
  - Apply precision / accuracy acceptance criteria
- Dilution with control matrix extract
- Recovery & suppression
- Assessment of indicating papers

The use of indicating papers



Accuracy, Precision & Sensitivity

DPS 226 paper
3 runs

Nominal concentration (ng/mL)	0.2	0.8	10	160	200
Mean concentration (ng/mL)	0.22	0.81	9.53	167.26	212.59
SD.	0.03	0.08	0.67	11.21	5.88
Overall precision (%CV)	13.2	10.1	7.0	6.7	2.8
Average accuracy (% bias)	9.0	0.9	-4.7	4.5	6.3
Average intra-run precision (%)	12.9	9.0	7.4	6.9	2.5
Inter-run precision (%)	3.8	5.4	Negligible	Negligible	1.4

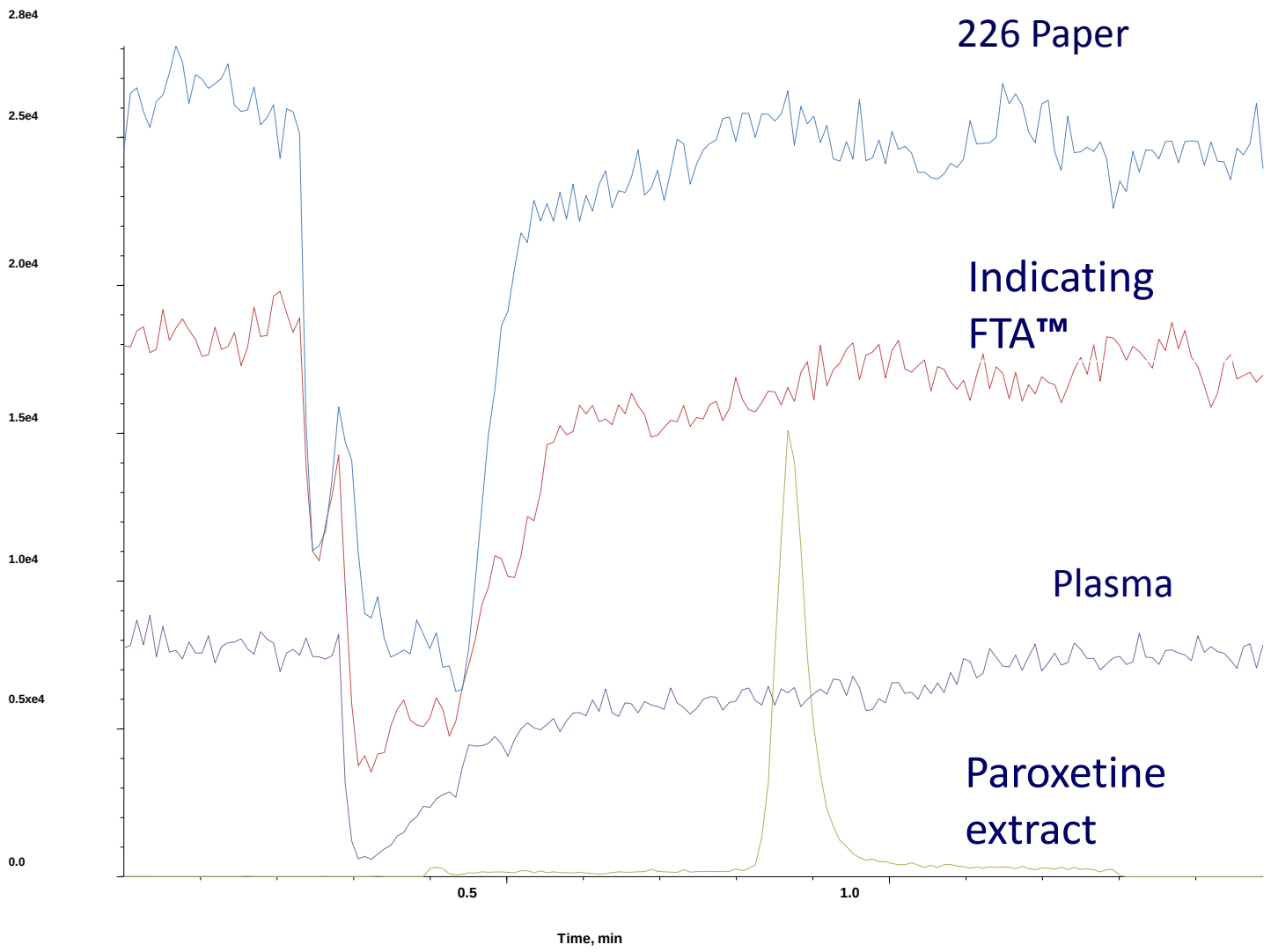
A developmental grade of untreated FTA™ DMPK
1 run

Nominal concentration (ng/mL)	0.2	0.8	10	160	200
mean concentration (ng/mL)	0.18	0.88	9.60	161.82	199.51
SD.	0.01	0.05	0.63	9.14	11.13
precision (%CV)	6.4	5.9	6.5	5.6	5.6
accuracy (% bias)	-10.7	10.2	-4.0	1.1	-0.2

Plasma spot size using 226

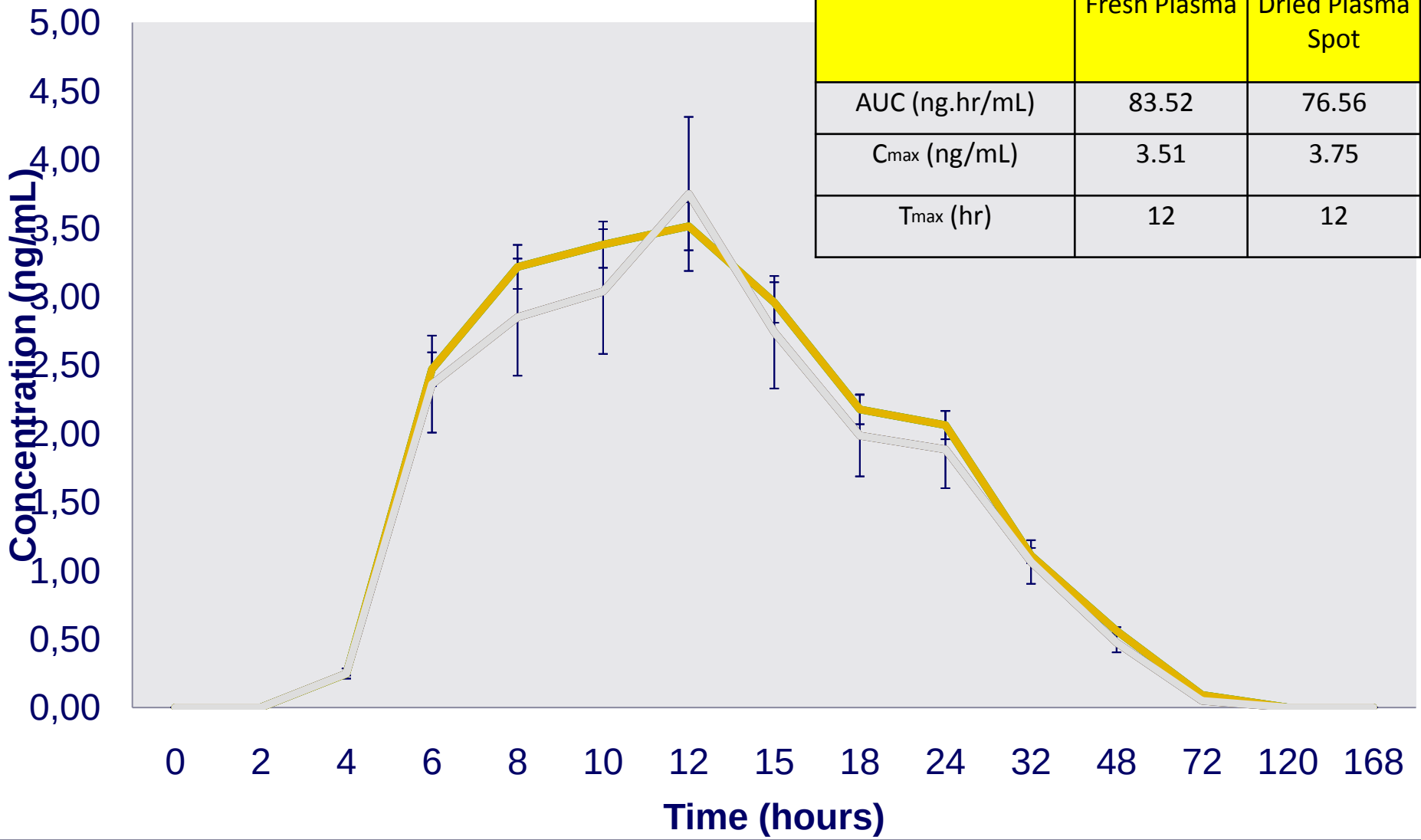
Nominal concentration	0.8 ng/mL			160 ng/mL		
Volume of human blood spotted onto FTA paper	15 µL	20 µL	25 µL	15 µL	20 µL	25 µL
Mean concentration (ng/mL)	0.76	0.83	0.76	167.0	172.8	167.7
SD.	0.061	0.076	0.042	6.4	7.8	13.1
Precision (%CV)	8.13	9.13	5.59	3.80	4.50	7.80
Accuracy (% bias)	-5.5	4.0	-5.0	4.4	8.0	4.8
Difference from 20µL Spot (%)	-9.2		-8.7	-3.4		-3.0

Post column infusion



Real pooled sample comparison – DPS v Plasma

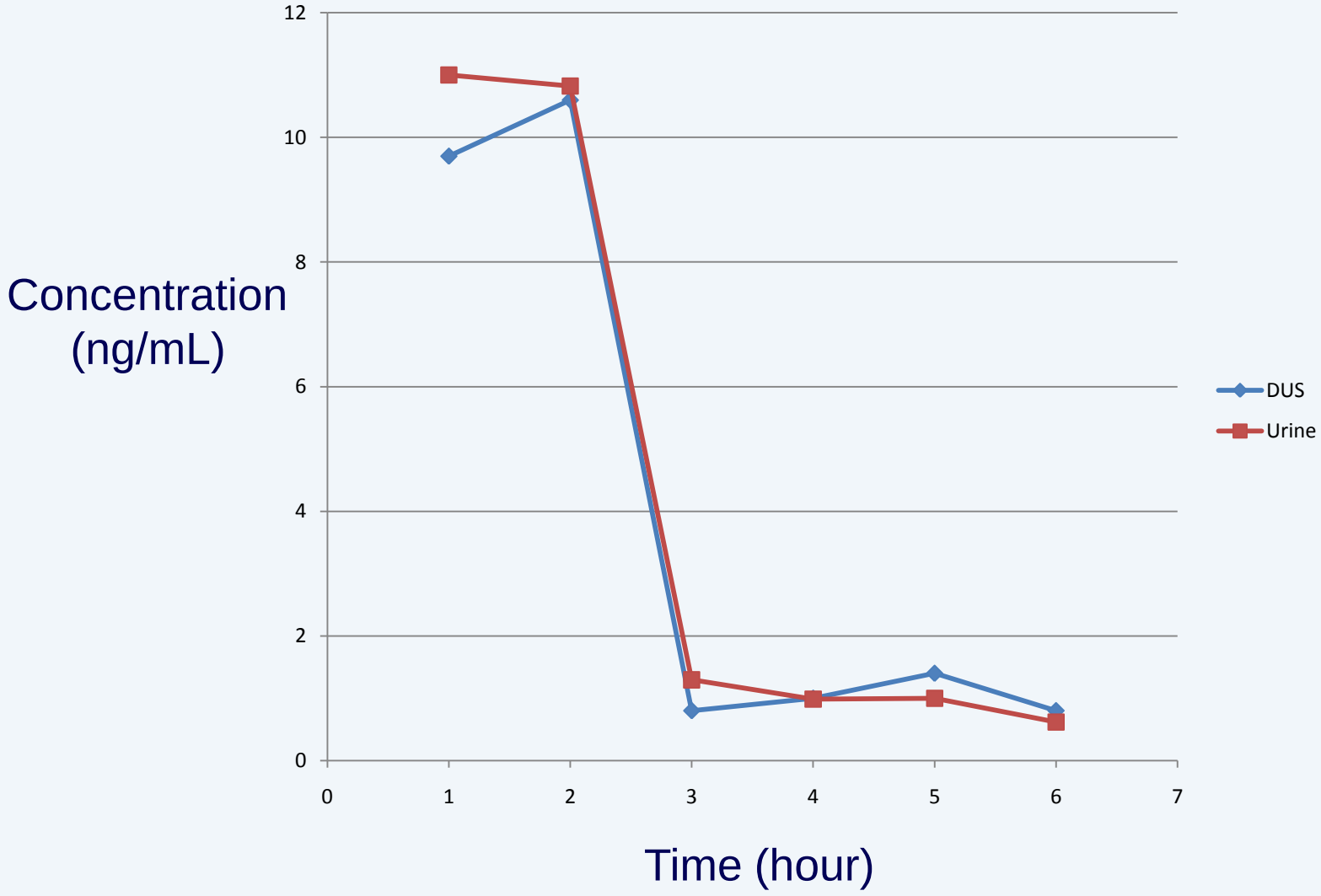
	Type of Sample	
	Fresh Plasma	Dried Plasma Spot
AUC (ng.hr/mL)	83.52	76.56
C_{max} (ng/mL)	3.51	3.75
T_{max} (hr)	12	12



DUS Data – precision & accuracy 1 run

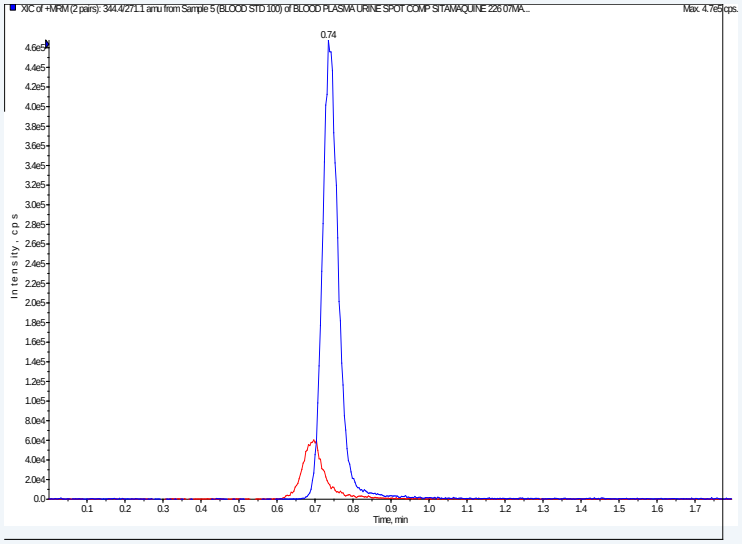
Concentration (ng/mL)	Mean	Standard Deviation	%CV	Accuracy
0.1	0.09763	0.007428	7.6	97.6
0.4	0.377821	0.022761	6.0	94.5
5	4.720766	0.379967	8.0	94.4
40	36.509379	1.787507	4.9	91.3
50	48.024223	1.244762	2.6	96.0

Real pooled sample comparison – DUS v Urine

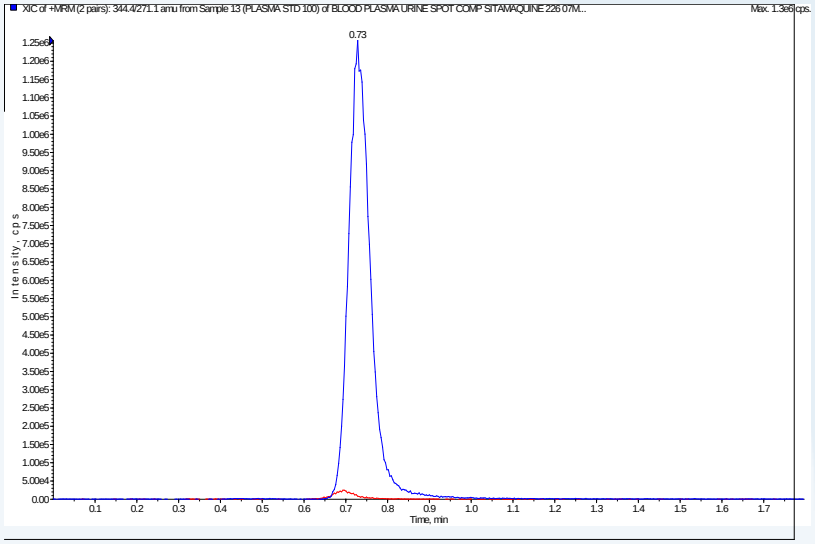


Sitamaquine : Direct Elution and Manual extraction

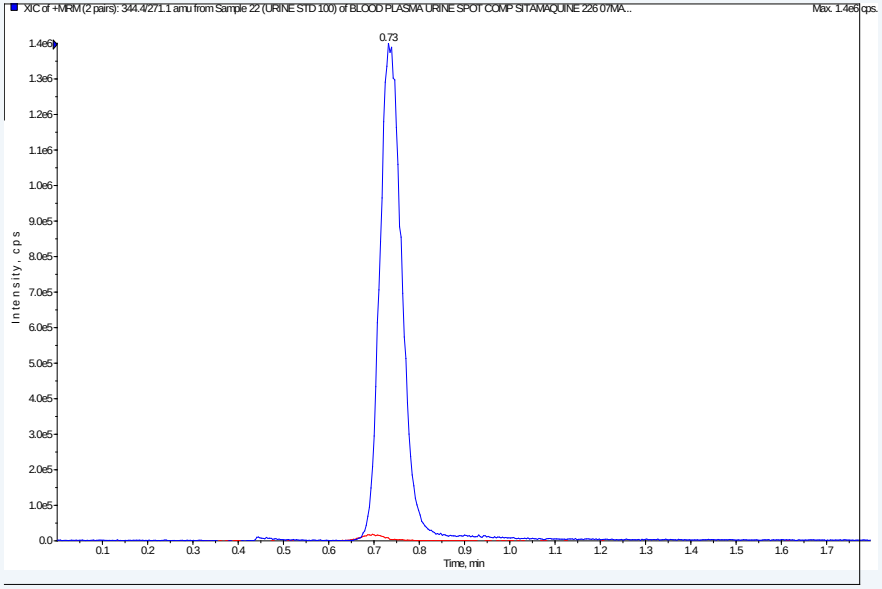
Blood



Plasma

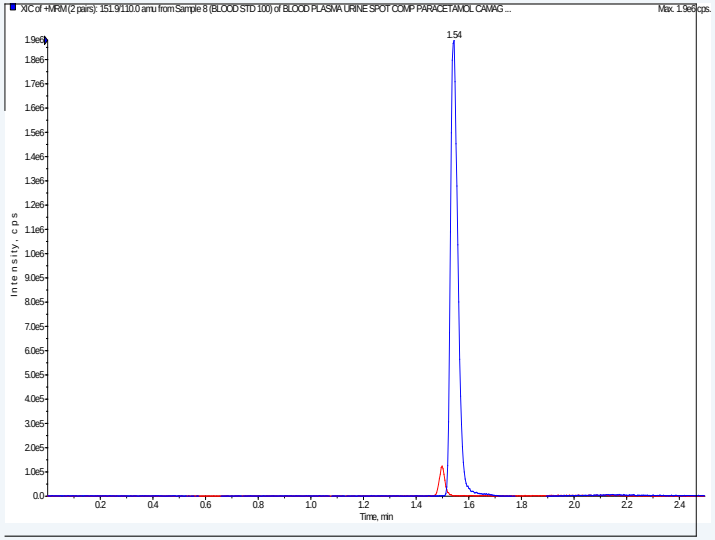


Urine

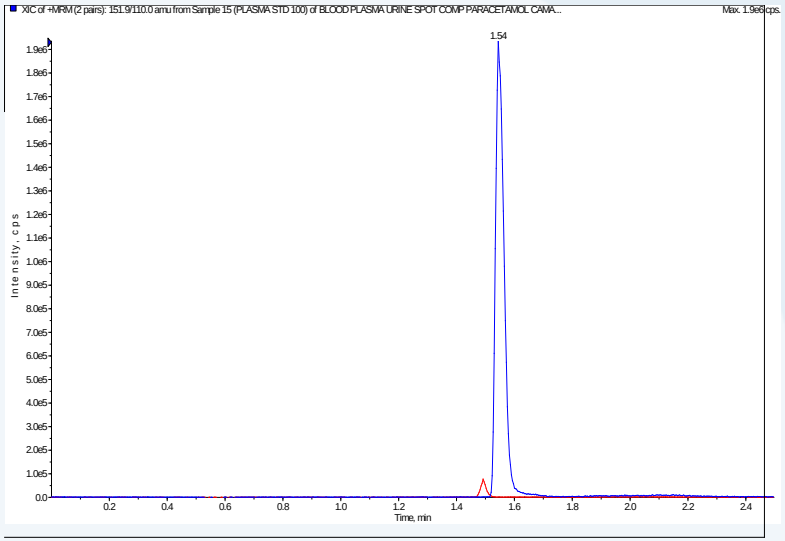


Paracetamol : Direct Elution and Manual extraction

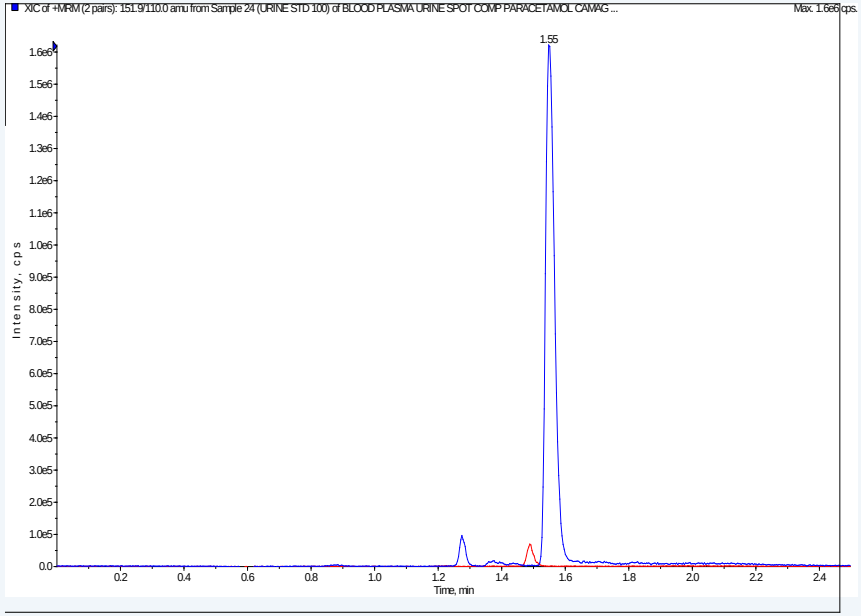
Blood



Plasma



Urine



Summary

- The use of DPS and DUS could accelerate the cost benefit offered by card technologies
- Many of the benefits of DBS apply to DPS and DUS
- DBS is still the preferred option but for late stage compounds and compounds that are not applicable for DBS then DPS has its uses
- DUS in my opinion is better scientifically and practically than urine
- Both DUS and DPS can utilise new technologies which offer advantages to the bioanalyst

Acknowledgements

GSK

- Neil Spooner
- Paul Abu-Rabie
- Rob Wheller

GE

- Mark Green