



The Use of Dried Blood Spot Samples for Quantitative Drug Bioanalysis: Implementation and Future Directions

Neil Spooner

PCD DMPK, GlaxoSmithKline, Ware, UK

(Neil.Spooner@gsk.com)

What are Dried Blood Spots? (DBS)

- Technique has been around for >40 years
- Easy way of collecting, shipping & storing blood samples
- Widely used in new born screening, therapeutic drug monitoring & for trials in remote areas, e.g. anti-malarials



Advantages of DBS for Non-Clinical

- Ethical benefits of reduced blood volumes (typically 3 x 10-20 µL blood spots)
 - Refinement
 - Elimination / reduction of rodent warming
 - Reduction
 - Serial sampling from one rodent rather than composite bleeds from several
 - Reduction / removal of satellite rodents
- Data quality
 - Serial vs. composite
 - Potential to obtain TK from 'main' study animals
- Enables juvenile studies
- Costs
 - Animal numbers
 - Procedures
 - Test substance

Advantages for Clinical

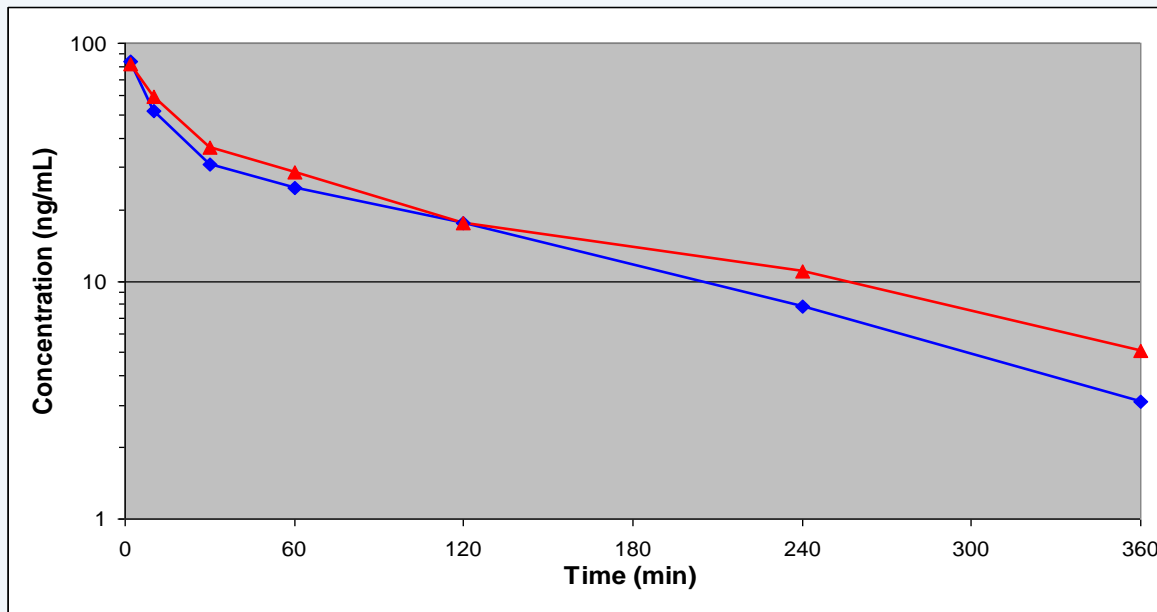
- Reduced blood volume
 - Enables paediatric studies
 - Advantages for critically ill patients
- Simplified blood sampling (finger prick)
 - Improved recruitment (Population Pharmacokinetics)
 - Ideal for Phase II/III in developing countries
 - Findings from GSK pilot clinical study
 - All staff (14) found blood spotting to be easy
 - 10 out of 11 subjects preferred finger prick to venous cannula
 - 2/3 finger pricks per timepoint (if required)
 - >6 samples per session / visit

Other Advantages

- Simplified process – better quality
 - ✘ Centrifugation
 - ✘ Sub-aliquotting
 - ✘ Freezing
 - ✘ Defrosting
- Reduced costs
 - Shipping – non-hazardous (inactivates HIV & Hep B)
 - Storage
- Potential for greater compound/metabolite stability
- *Increased communication with customers!*

Validating the Technology (1)

- Initially 10 structurally diverse compounds were validated
 - Precision & accuracy
 - On card stability
 - Assay ruggedness to pipetting error
- 12 additional compounds then tested in rats
 - Direct comparison of conventional technique (blood:water 1:1 v/v) vs DBS using in-vivo samples

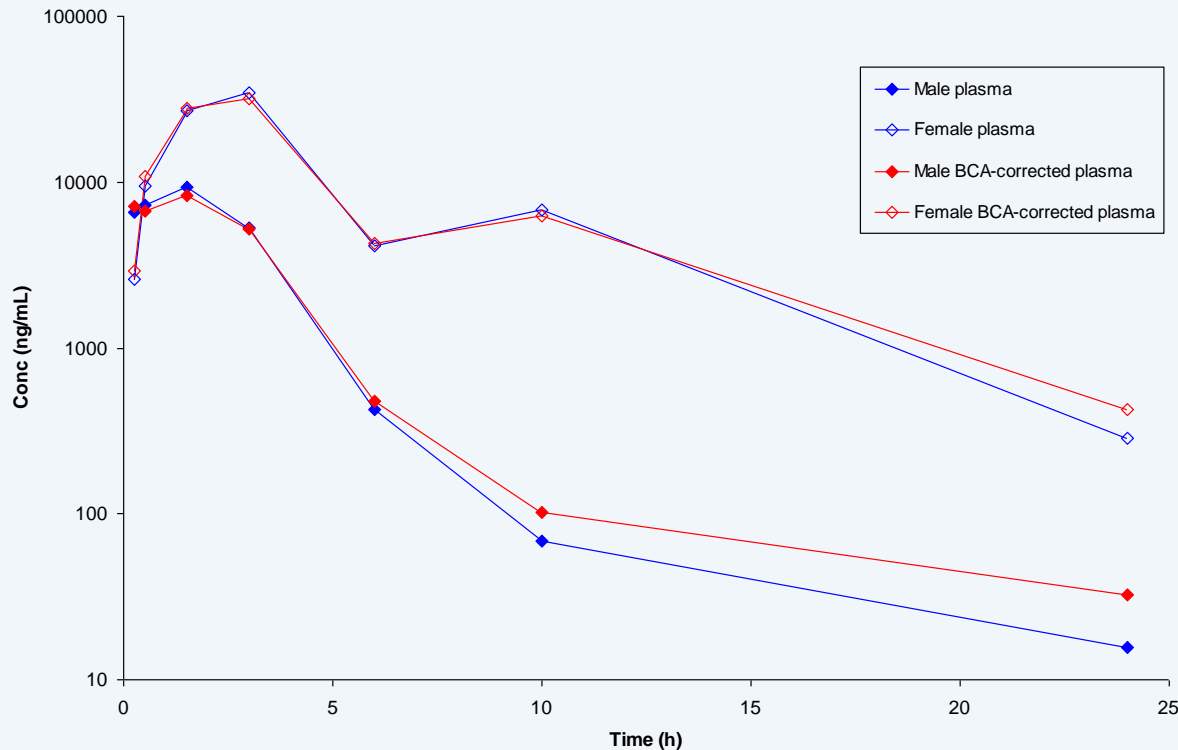


Blood:water & DBS

Validating the Technology (2)

- Pilot *in vivo* Safety Assessment studies started in January 2007
 - nonGLP 7 day studies
 - Plasma primary data
 - DBS and blood/water samples also taken
 - All pre-clinical Sites involved

Day 1 data at 600mg/kg



GSK's Strategy

- World Wide agreement & implementation
 - DBS adopted as preferred technique for assessment of TK of all new oral compounds selected as candidates, where a bioanalytical method has been validated
 - Once we commit to a matrix at pre-candidate selection that matrix will not change throughout the life of the compound, including Clinical phases
 - Switch to DBS for late stage compounds only considered for particular circumstances, e.g. paediatrics, critically ill patients, drugs of the developing world, population PK

Status of DBS Sampling at GSK*

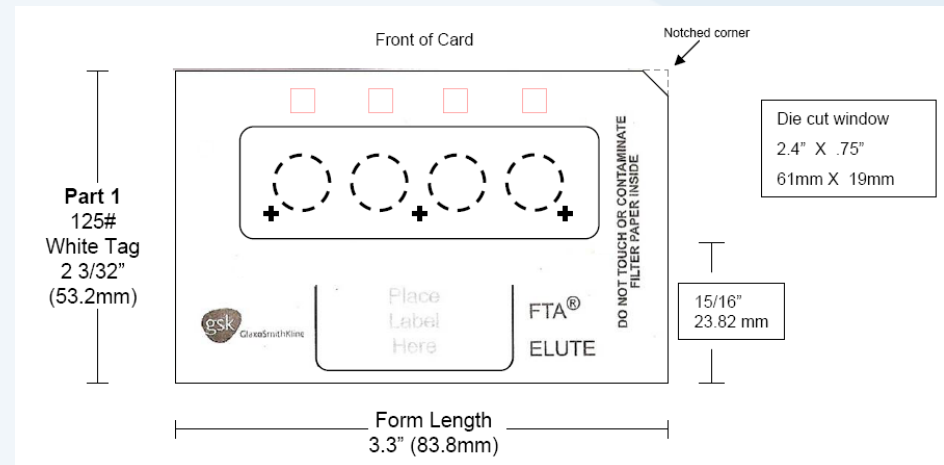
- 153 DBS bioanalytical methods validated for 90 compounds
- Studies **Completed**

	Studies	Analytes	Samples
Pre-Clinical nonGLP	152	79	24,733
Pre-Clinical GLP	68	29	20,087
Clinical	7	11	5,571

* as of 29 Jan 2010 (does not include incurred sample reanalysis)

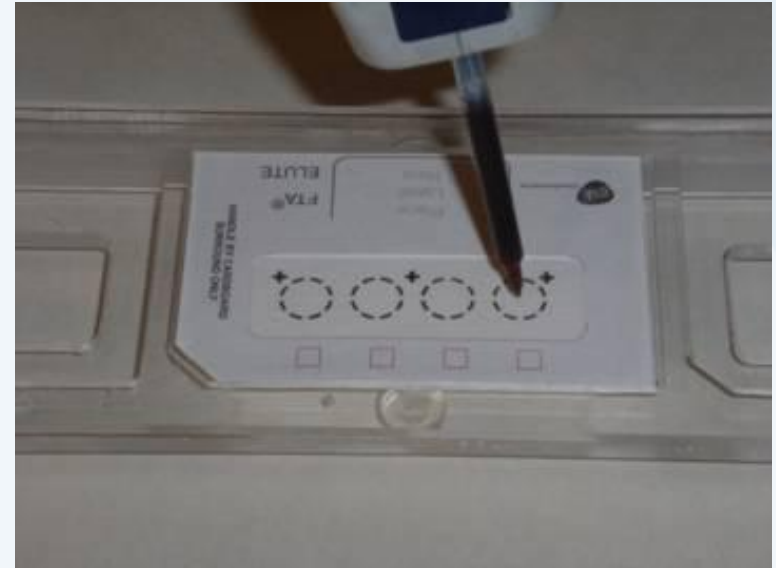
Blood Spot Card

- Currently use 3 types of paper
 - Whatman FTA DMPK A (FTA®) & B (FTA® Elute)
 - Both contain additives (designed for nucleic acid analysis)
 - Ahlstrom 226
 - Untreated
- Whatman FTA DMPK C (31ETF; untreated) also available
- Guthrie / 903 (untreated) paper
 - Uniformity issues



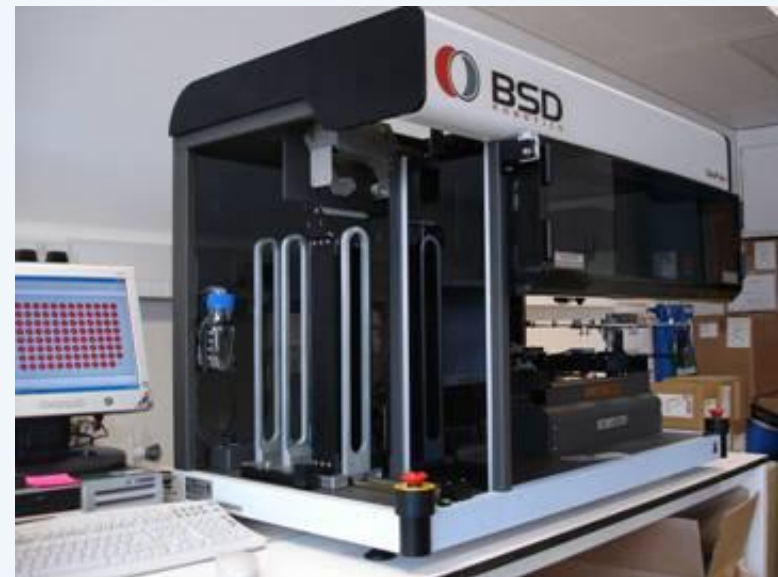
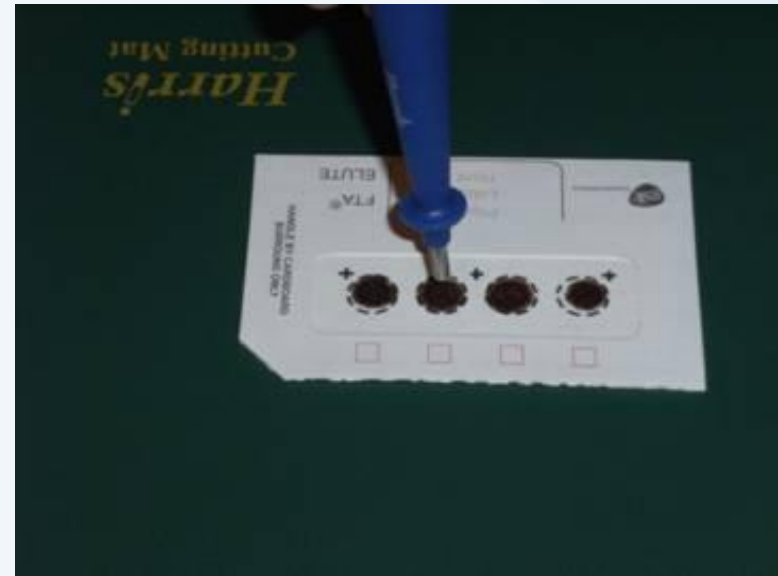
Blood Spotting

- Aliquot 15 μL blood per spot
 - 3 spots per sample plus 'spare'
 - Using a pipette or capillary
 - Do **NOT** allow tip to touch card surface!
- Dry for ≥ 2 hours at room temperature
- Ship & store in sealable bags containing desiccant



Sample Prep & Analysis

- Analytical methods for DBS samples are validated to internationally accepted criteria
 - Including Incurred Sample Reanalysis (ISR)
- Analytical sample obtained by punching small disc (typically 3 mm) from centre of DBS
 - This step is analogous to the aliquot removal during processing of liquid samples
- Extract disc in organic solvent (typically methanol) containing internal standard
- Quantitation by LC-MS/MS



Dried Blood Spots – Next Steps for Bioanalysis!

- Although benefits of DBS sampling to drug development are now starting to be realised.....
- The approach is more difficult for the bioanalyst
 - More complex method development, validation & sample analysis than plasma
 - Multiple card types
 - More variability in extraction solvents
 - Punching
 - Non-optimised automation
 - Lower sensitivity
 - Increased ion suppression
 - etc.....

What Do We Do About It Then?

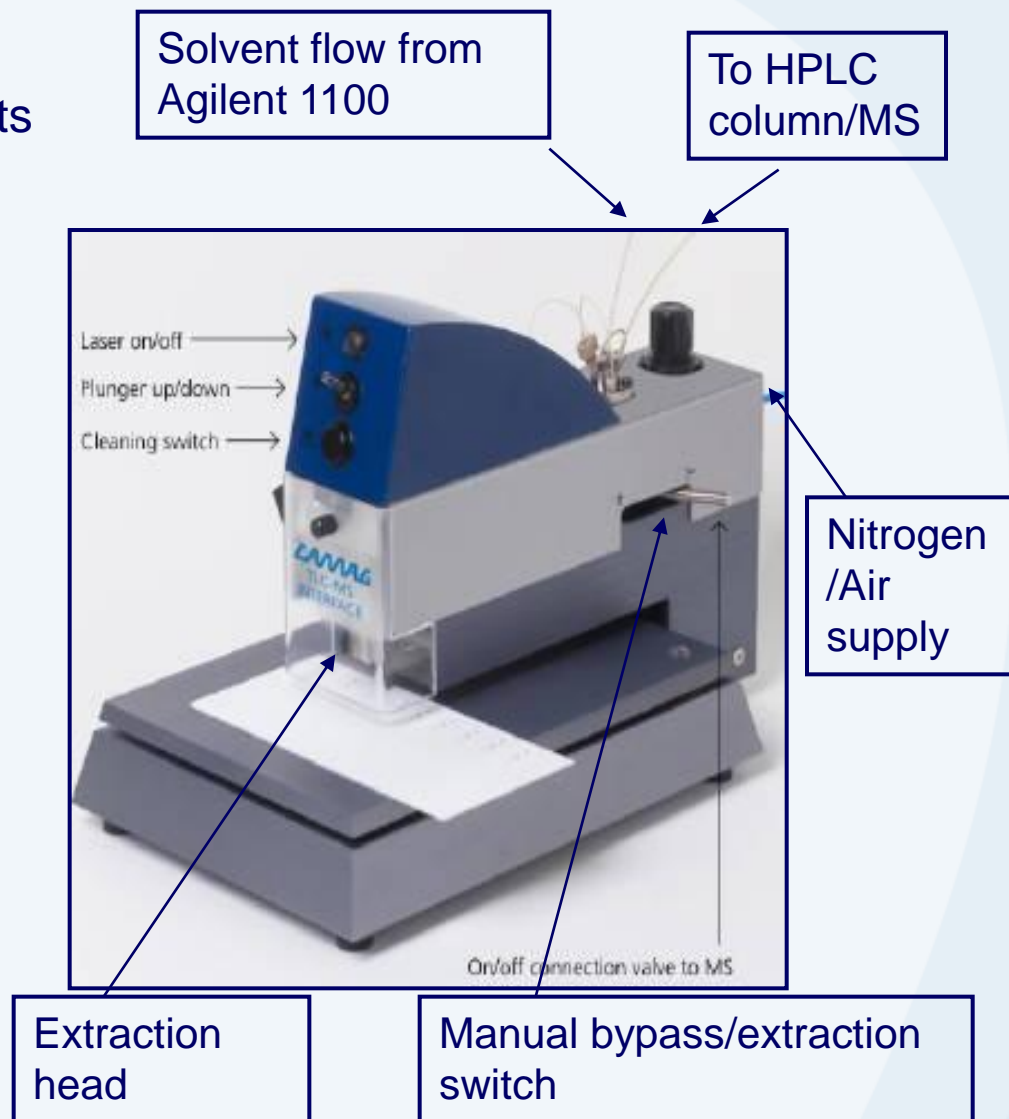
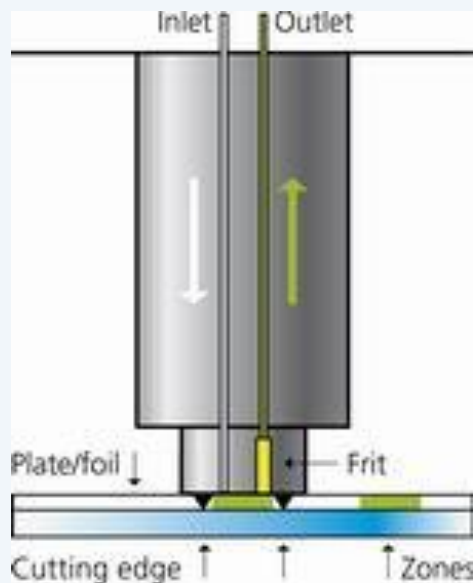
- Short term
 - Investigate the parameters of DBS sampling
 - Hematocrit, lipidemic, anticoagulant, etc
 - Storage of cards
 - Paper uniformity
 - Age of control blood
 - Drying time
 - Homogeneity
 - New paper types
 - Device for drying / transport / storage / interface with sample analysis
 - Plastic mounts
 - More robust
 - Protection from contamination
 - Improved automation
- Longer term
 - Direct analysis of blood spot samples

Direct Analysis of DBS Samples

- First step is to get analyte off the cards and ionised in the gas phase
- A number of approaches are being developed, including, but not limited to.....
 - Direct elution
 - University Center of Legal Medicine, Switzerland - On-line desorption
 - CAMAG - TLC-MS Interface
 - Spark Holland
 - Advion – TriVersa NanoMate
 - Direct desorption
 - Prosolia & Purdue - DESI
 - IonSense - DART
 - Purdue - Paper spray
 - Universita di Foggia & Universita Federico II di Napoli, Italy - Atmospheric pressure thermal desorption chemical ionisation

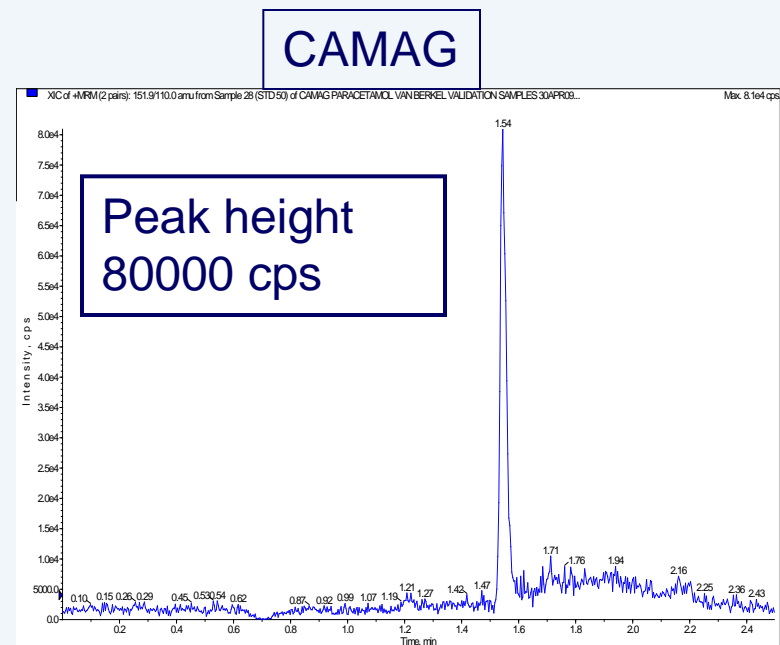
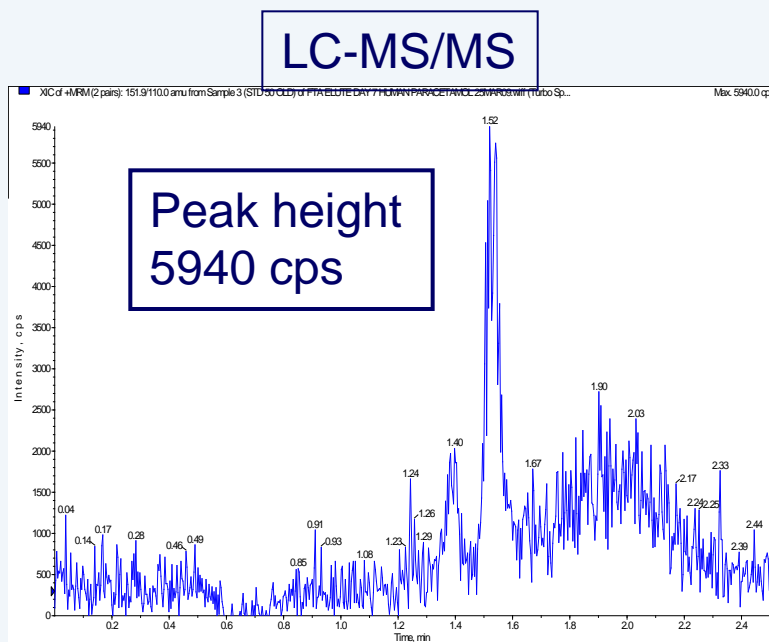
CAMAG TLC-MS Interface

- Extracts 4 mm diameter zone
- Uses standard HPLC-MS solvents & flow rates
- Online transfer into the MS
- Automatic cleaning of piston between extractions



CAMAG DBS - Comparative Sensitivity Test

- Acetaminophen DBS at 50 ng/mL (LC-MS/MS assay LLQ)
- **Validated LC-MS/MS** method, 2 μ L inj (from 100 μ L), gradient LC
- **CAMAG**, 2 second extraction, chromatography conditions as validated method (2.5 min run time)



TLC-MS increases acetaminophen assay sensitivity by a factor of ~13

CAMAG DBS – Sensitivity vs Manual Extraction

- All DBS samples on untreated paper (226)
- All CAMAG data acquired after 2 sec extraction with mobile phase & same LC method as used for manual DBS extraction
- Manual extraction data all for 3mm punch from DBS extracted with 100 μ L solvent

	Increase in MS response / Fold	Theoretical Maximum Increase in Response / Fold	Measured Increase in Response versus Theoretical Maximum / %
<i>Single Analyte Assays</i>			
Sitamaquine	16	36	45
Paracetamol	7	89	8
<i>Cassette Test Compounds</i>			
Ibuprofen	11	89	12
4-Nitrophthalic Acid	8	89	9
Paracetamol	5	89	6
Simvastatin	13	89	15
Sitamaquine	14	89	16
Benzethonium Chloride	4	89	4
Proguanil	15	89	17

CAMAG DBS - Accuracy & Precision

Sitamaquine

Nominal QC Conc' (ng/mL)	Mean Measured Conc' (ng/mL)	Precision (% CV)	Accuracy (%)
5	5.2*	5.5*	104.7*
20	20.9	10.2	104.4
100	110.7	13.2	110.7
800	883.7	3.0	110.5
1000	1101.7	1.1	110.2

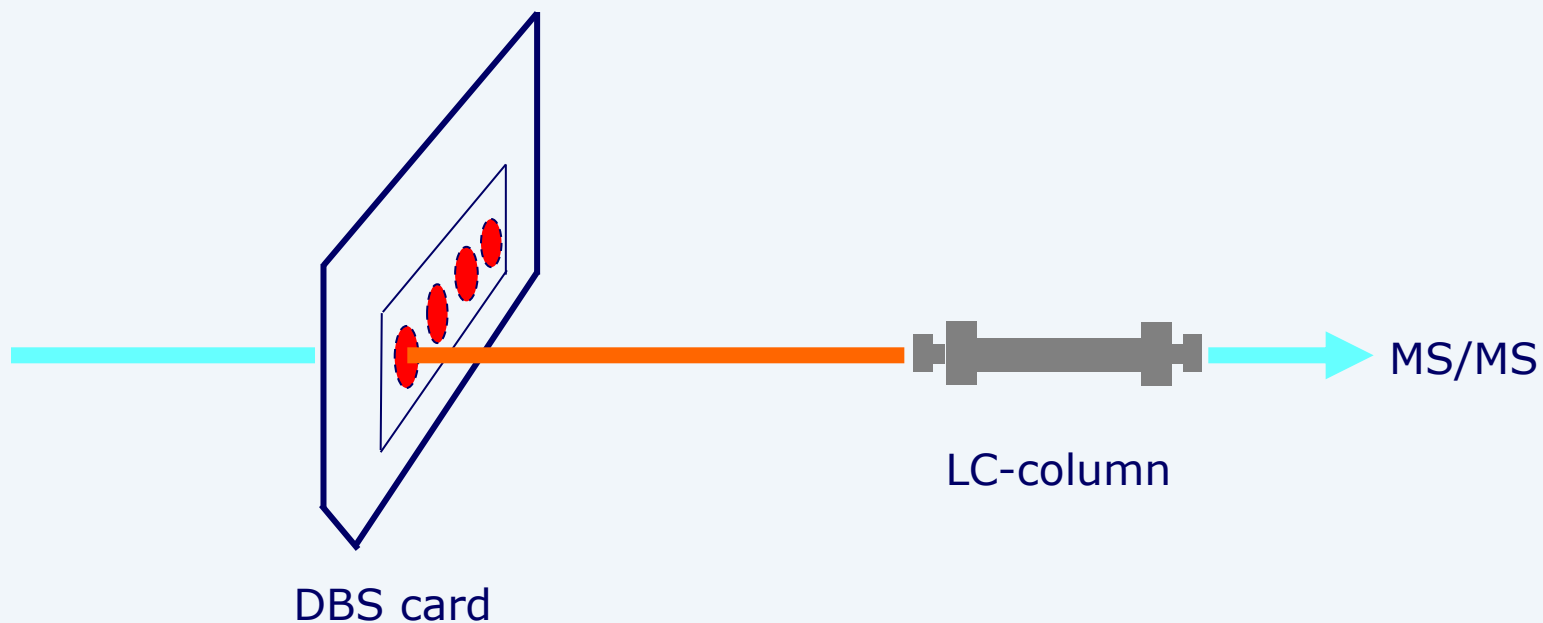
Acetaminophen

Nominal QC Conc' (ng/mL)	Mean Measured Conc' (ng/mL)	Precision (% CV)	Accuracy (%)
50	112.3	20.7	224.6
200	202.5	12.8	101.3
5000	5299.5	6.8	106.0
40000	39723.6	9.9	99.3
50000	52268.8	4.1	104.5

n=4 values

*n=6 values after back-flush of extraction head

Spark Holland Prototype - DBS Liquid Extraction Sealing Surface Sampling



Spark Holland Prototype Data

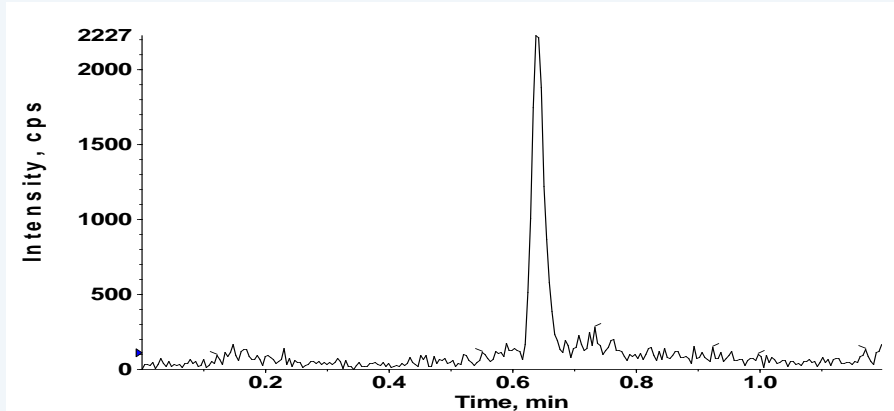
- Retains retention and chromatographic performance for all of our 'chemical space' compounds
- Increase in assay sensitivity
- Investigated elution to loop:
 - Sitamaquine DBS (untreated paper) eluted using 35 μ L methanol:water (50:50 v/v)
 - Extract transferred to loop
 - Injection from loop using standard mobile phase

Nominal Conc' (ng/mL)	Measured Conc' (ng/mL)	Precision (%CV)	Accuracy (%)
5	5.07	2.4	1.3
20	19.83	0.6	-0.9
100	94.08	1.5	-5.9
800	740.48	1.7	-7.4
1000	860.93	1.8	-13.9

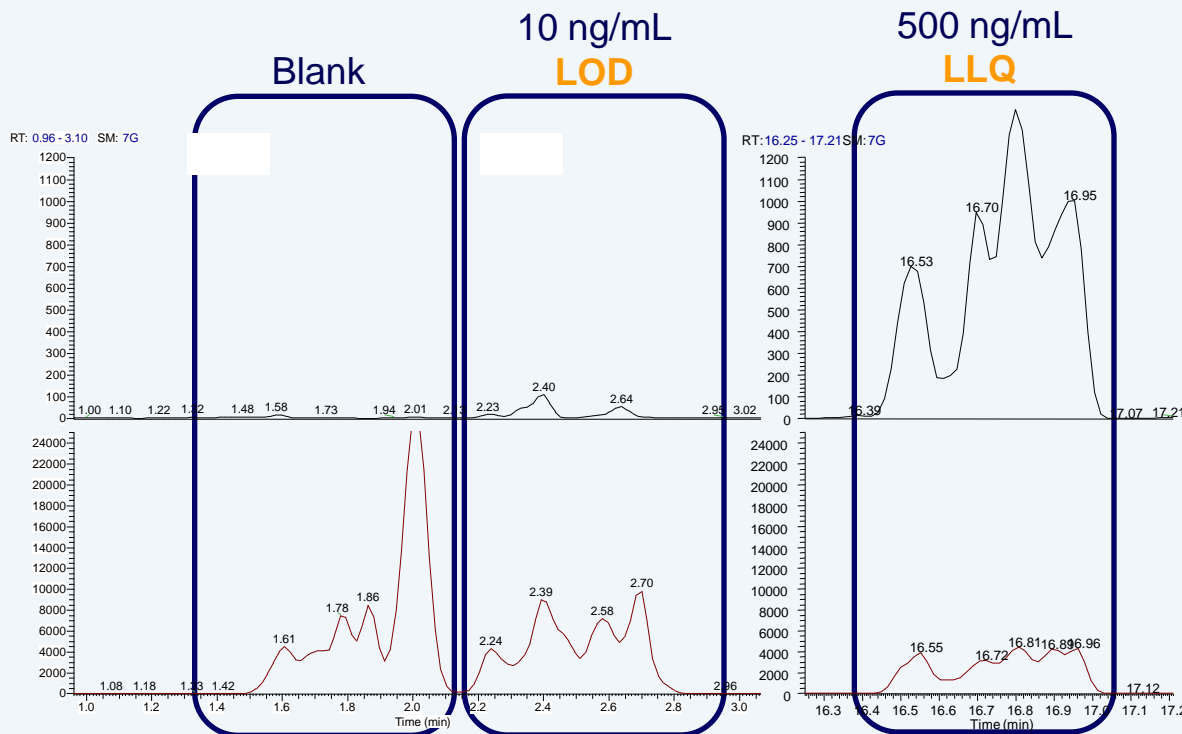
n = 6 values

Validated Run Data – LC-ESI vs. DESI

Sitamaquine



LC-ESI-MS/MS
Chromatogram at
10 ng/mL- LLQ
(API5000)



DESI-MS/MS
Chromatogram

IS
(550 ng/mL)

Validated Run Data – LC vs. DESI

LC-MS/MS Data

Sitamaquine QC Concentration (ng/mL) from 3-Day Validation					
	10	40	500	8000	10000
Mean	10.2	40.3	490.9	7690.7	9849.8
SD	0.8	3.2	27.1	487.8	523.9
CV (%)	7.4	8.0	5.5	6.3	5.3
Bias (%)	1.7	0.6	-1.8	-3.9	-1.5
n	18	18	18	18	18

Range 10 – 10000 ng/mL
 $1/x^2$ linear regression
 $Y = 0.00112x + 0.0029$ ($r=0.9991$)

DESI-MS/MS Data

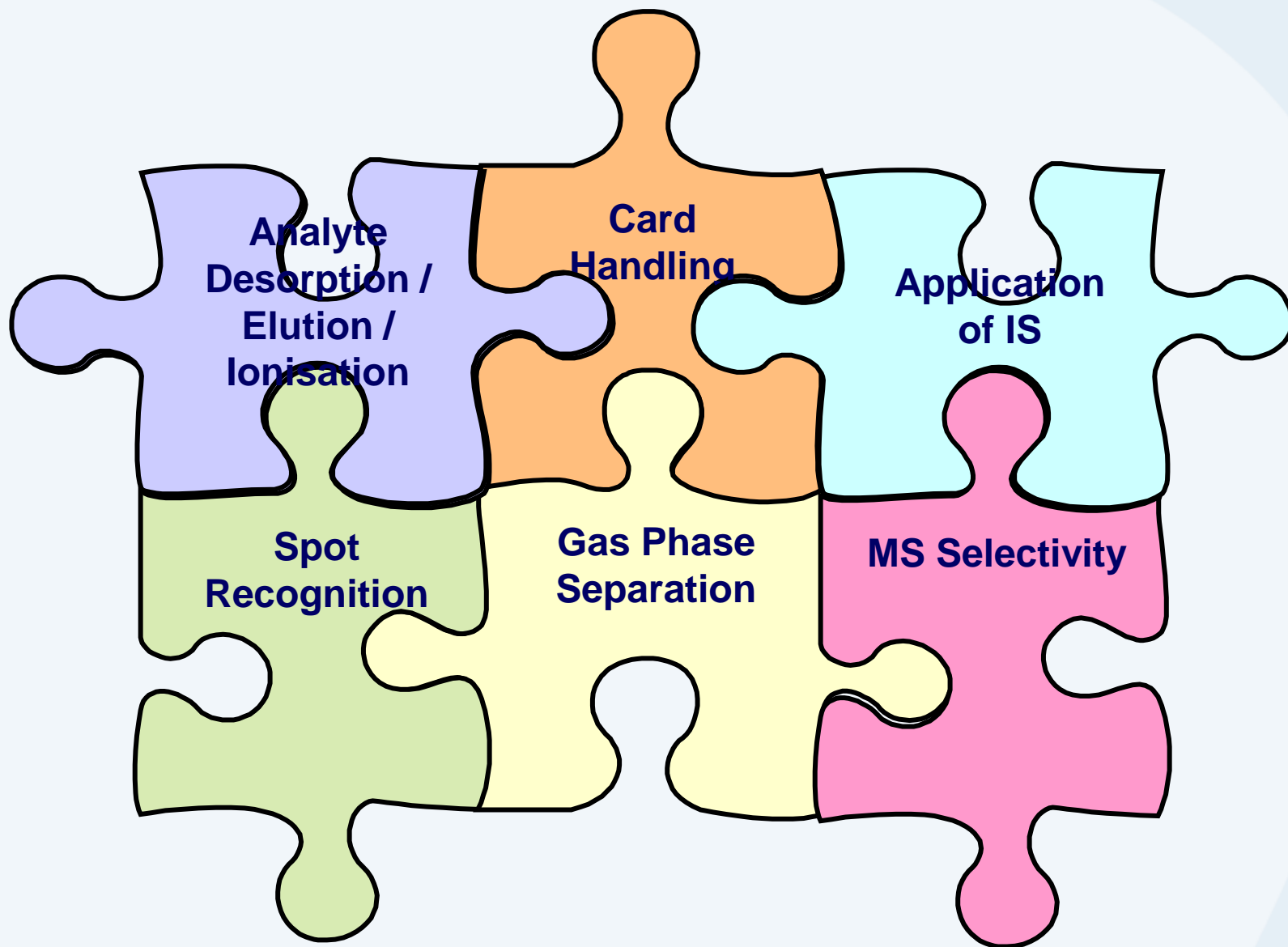
Sitamaquine QC Concentration (ng/mL)					
	10	40	500	8000	10000
Mean	41.9	58.8	456.4	8737.8	10271.3
SD	7.0	14.2	66.5	1099.7	1593.6
CV (%)	16.8	24.1	14.6	12.6	15.5
Bias (%)	319.4	47.0	-8.7	9.2	2.7
n	6	6	6	6	6

Range 10 – 10000 ng/mL
 linear regression
 $Y = 0.0005x - 0.0168$ ($r=0.9991$)

It's Not Just About Making & Detecting Ions!

- Need to have an IS
 - Correct for variability in sampling, recovery, uneven surface, ionisation, etc
- Have to handle cards
 - Present samples to interface
 - Spot recognition
 - Repeats
- Can't dilute samples
 - Need detector with wider dynamic range
 - 5 orders of magnitude?
- What about interferences?
 - Unstable conjugated metabolites
 - O-glucuronides, N-glucuronides, N oxides, sulfates
 - Ion suppression
 - Could ion mobility / FAIMS help?
- If LC eliminated, do we have enough selectivity?
 - Could mass resolution, MS^n help?

Putting Together the Pieces



Putting Together the Pieces

- A number of approaches have demonstrated that the direct analysis of DBS is possible
 - Acceptable sensitivity, linearity, precision & accuracy
- All the potential parts of the jigsaw are currently available
- The challenge is to turn these different parts and prototypes into robust instruments that can be used in a working laboratory
 - Ease of use
 - Reliability
 - Affordable
 - Service provision
 - User support
 - etc.

A New MS Paradigm for Quan MS?!

- Could the direct sampling of DBS lead to the replacement of the triple quad as the standard platform for quantitative bioanalysis?
- Potential to simplify MS design & use
 - Interface may not need to work for high liquid volumes
 - Selectivity obtained in ways other than MRM
 - Reduced instrument optimisation / tuning
 - True 'walk up' instrumentation?
- The above has the potential to refresh existing instrumentation markets (e.g. Pharma) & open-up new markets
 - De-centralisation of analysis?
 - Hospital labs
 - Therapeutic drug monitoring
 - Forensics
 - Pharmacodynamic markers
 - Biomarkers / metabonomics
 - Blood chemistry
 - etc.....

Acknowledgements

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 - Bert Ooms
 - Emile Koster
- Whatman
 - Mark Green
- ID Biological
 - John Dinan
 - Brad Davin

Upcoming Events Focusing on DBS for PK & TK

- Dedicated session at [ASMS 2010](#) – Salt Lake City, 23–27 May 2010
 - Focus on direct analysis of DBS samples
- [EBF Meeting 'Connecting Strategies on Dried Blood Spots'](#) - Brussels, 17-18 June 2010
 - Covering all aspects associated with the implementation of DBS sampling
 - Safety Assessment
 - Bioanalytical
 - Clinical
 - Sample Management
 - Regulatory
 - Etc.....
- Dedicated volume of [Bioanalysis Journal](#) – August & October 2010
- Presentations and Discussion at the [27th Montreux LC-MS Symposium](#) - Montreux, 10-12 Nov 2010
- Dedicated session at [AAPS 2010](#) – New Orleans, 14-18 Nov 2010

Useful Publications

- M Barfield, N Spooner, R Lad, S Parry, S Fowles (2008) *J. Chromatogr. B* **870**, 32-37
Application of dried blood spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicology studies
- N Spooner, R Lad, M Barfield (2009) *Anal. Chem.* **81**, 1557-1563
Dried blood spots as a sample collection technique for the determination of pharmacokinetics in clinical studies: considerations for the validation of a quantitative bioanalytical method
- W Li, LS Tse (2010) *Biomed. Chromatogr.* **24**, 49-65
Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules
- J Deglon, A Thomas, A Cataldo, P Mangin, C Staub (2009) *J. Pharm. Biomed. Anal.* **49**, 1034-1039
On-line desorption of dried blood spot: A novel approach for the direct LC/MS analysis of μ -whole blood samples
- P Abu-Rabie, N Spooner (2009) *Anal. Chem.* **81**, 10275-10284
Direct quantitative bioanalysis of drugs in dried blood spot samples using a thin-layer chromatography mass spectrometer interface

Useful Publications Cont'd

- V Kertesz, GJ Van Berkel (2009) *J. Mass Spectrom.* **45**, 252-260
Fully automated liquid extraction-based surface sampling and ionisation using a chip based robotic nanoelectrospray platform
- JM Wiseman, CA Evans, CL Bowen, JH Kennedy (2010) *Analyst* **135**, 720-725
Direct analysis of dried blood spots utilizing desorption electrospray ionisation (DESI) mass spectrometry
- G Paglia, O D'Apolito, M Gelzo, A Dello Russo, G Corso (2010) *Analyst* **135**, 789-796
Direct analysis of sterols from dried plasma/blood spots by an atmospheric pressure thermal desorption chemical ionisation mass spectrometry (APTDCI-MS) method for a rapid screening of Smith-Lemli-Opitz syndrome
- H Wang, J Liu, RG Cooks, Z Ouyang (2010) *Angew. Chem. Int. Ed.* **49**, 877-880
Paper spray for direct analysis of complex mixtures using mass spectrometry



Backup Slides

Regulatory Perspective

- Blood v plasma
- Blood data has already supported regulatory filings

ICH S3A says“The quantification of systemic exposure provides an assessment of the burden on the test species and assists in the interpretation of similarities and differences in toxicity across species, dose groups and sexes. The exposure might be represented by plasma (serum or **blood**) concentrations or the AUCs of parent compound and/or metabolite(s).”

.....The choice of analyte and the matrix to be assayed (biological fluids or tissue) should be stated and possible interference by endogenous components in each type of sample (from each species) should be investigated. Plasma, serum or **whole blood** are normally the matrices of choice for toxicokinetic studies”

Status of DBS Sampling at GSK – Methods

- LLQs based on Project requirement, not limitations of DBS approach
- Plasma data for 907 validated assays
- DBS not used for respiratory indications (low LLQs)

	% of Total Assays	
Assay LLQ (ng/mL)	Dried Blood Spot	Plasma
0.1 – 1	12	36
1.1 - 20	54	46
21 – 50	29	13
51 - 250	5	5

Current Components of GlaxoSmithKline Assay Validation

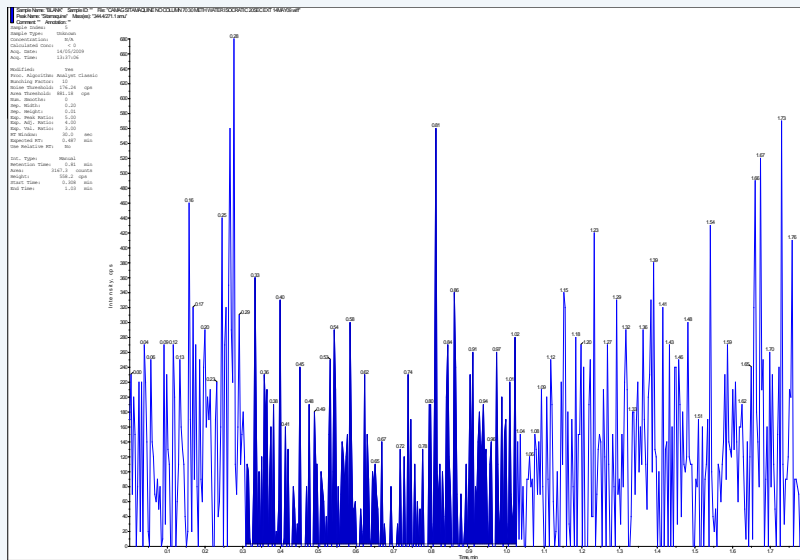
- Linearity
 - Duplicate calibration lines (front & back)
- Precision, accuracy, sensitivity & reproducibility
 - 5 concentrations, 6 replicates
 - 3 occasions for 1st pre-clin species & human
 - Single occasion for subsequent species
- Whole blood stability
 - 2 concentrations (VC2 & 4), 6 replicates at 37 C for 4 hrs
 - Precipitation of whole blood with solvent containing IS – compare peak areas to fresh
- On card stability
 - 2 concentrations (VC2 & 4), 6 replicates desiccated at room temp for ≥ 7 days
- Processed sample stability
 - Re-inject validation QCs with fresh calibrants after storage at room temp for ≥ 24 hrs
- ISR/ISS
- Selectivity
 - Total blanks & blanks from 6 different sources
- Assay robustness to pipetting error (10 - 20 μ L)
 - 2 concentrations (VC2 & 4), 6 replicates
 - Apply precision / accuracy acceptance criteria
- Dilution with control matrix extract
- Recovery & suppression

CAMAG DBS Without Column – Sensitivity

● Sitamaquine

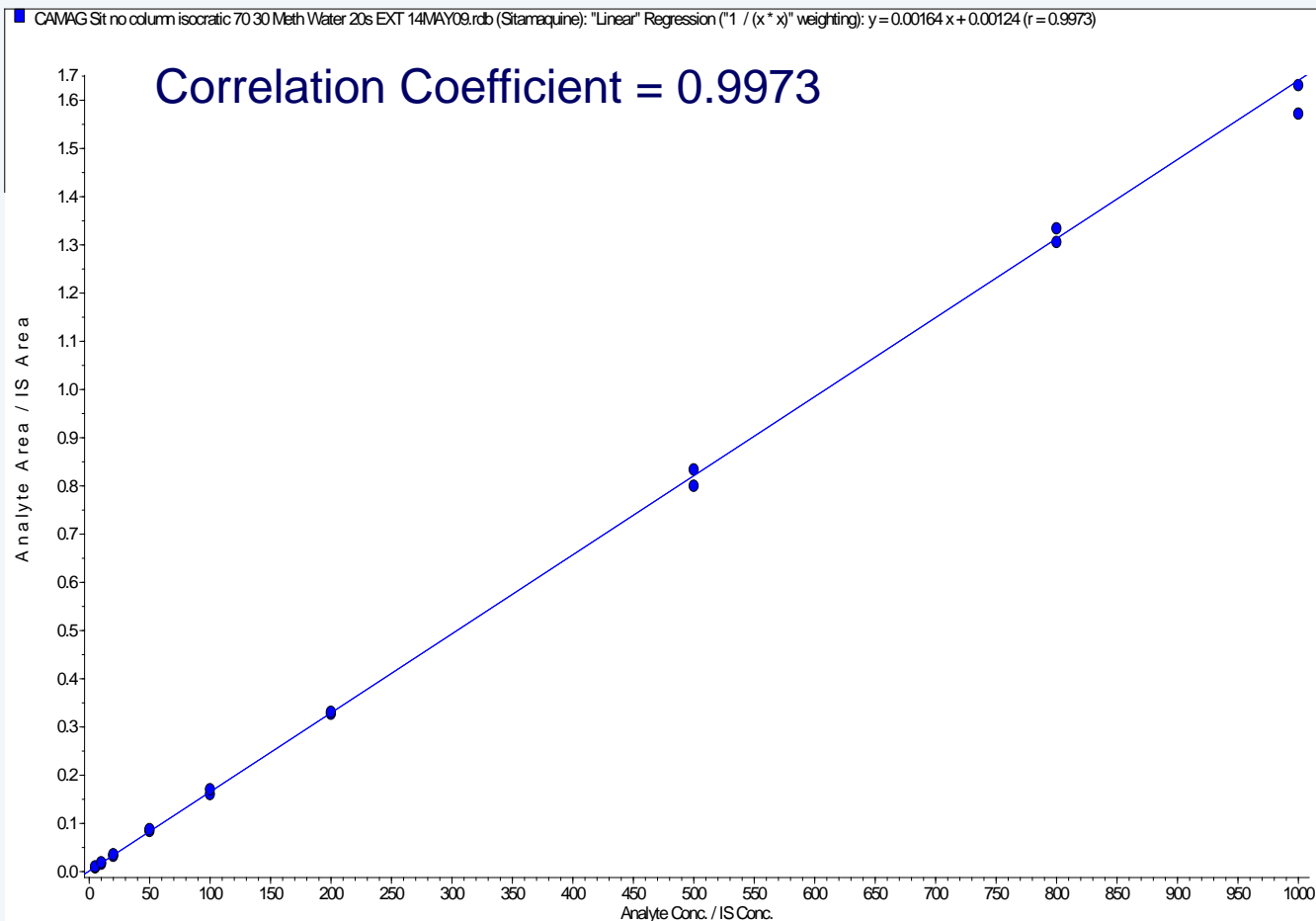
- DBS on untreated paper (5-1000ng/mL)
- 20 sec extraction, 1.8 min acquisition time
- 70:30 MeOH:H₂O (v/v)

Blank



CAMAG DBS Without Column – Linearity

Sitamaquine



CAMAG DBS Without Column – Precision & Accuracy

Sitamaquine

Nominal Conc' (ng/mL)	Mean Measured Conc' (ng/mL)	Precision (% CV)	Accuracy (%)
20	20.4	2.6	102.1
800	833.2	2.8	104.1

n = 6 values