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Dried Blood Spot sampling evaluated for pharmacokinetic and pharmacogenetic applications through a study with midazolam in healthy volunteers

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INTRODUCTION

Conventionally, plasma has been used for the determination of pharmacokinetics (PK) of new chemical entities in clinical trials because of its ease of handling, shipping, and storage compared to whole blood (WB). However, it is notable that regulatory authorities acknowledge that blood is an acceptable or perhaps even better biological matrix for the measurement of drug exposures. The collection of blood samples on paper, known as dried blood spots (DBS), is an upcoming technique for use in humans for therapeutic drug monitoring and PK studies. It offers a number of advantages over conventional plasma sampling, i.e. small blood volumes required for DBS samples, the technique being less invasive, more simple sample preparation and transfer and easy storage and shipment.
In addition to PK blood sampling, DBS has also been reported as sampling and sample storage tool for genotyping.
We see a large potential for DBS genotyping in a drug development setting as well, mainly for convenience reasons for subjects, ease of sample shipment and storage and more simple sample preparation procedures with DBS.
We here describe the conduct of an IRB approved open-label phenotyping and genotyping study in healthy volunteers with 7.5 mg midazolam tablets with the following objectives:

- Compare venous DBS and WB (1-OH-)midazolam results
- Compare finger puncture collected DBS and venous WB results
- Compare DBS and WB CYP3A4, 3A5, 2D6 and 2C19 genotyping results
- Evaluate subject and phlebotomist perception on finger puncture/DBS versus venous blood sampling

METHODS

Overall Study Design and Plan
- Study: one-period in 12 healthy subjects
- Medication: Midazolam tablet 7.5 mg administered 2h before a standard meal

Study Assessments
- Hematology, Blood chemistry, Virus serology and Urine Drug screen

Techniques for blood sampling
- Venous sampling (reference samples): venous blood was collected by venipuncture for WB PK (1st reference) (R1) and plasma PK (R2) and stored at -20°C. The collected WB was also pipetted on DBS cards as 2nd WB reference (R3)
- Finger puncture (test samples): blood was collected from finger puncture with an Accu check® safe-T-pro lancing device (2.3 mm depth). After wiping away the first drop, blood was sampled for DBS with an EDTA coated Bilbate® capillary (test sample, T) (Figure 1).
- Dried blood spots: DBS R3 and T were collected on IDBS Bioanalysis Card. The DBS cards were dried for 2 h at room temperature, and stored at room temperature.
- Total Blood Volume: 72 mL per subject during the entire course of the study, see table 1

Sample shipment and storage
- DBS cards were packed in plastic bags with desiccator; after the clinical phase all samples were sent to the laboratory

Pharmacokinetic assessments (PK)
- Midazolam and 1-OH-M were measured with LC-MS/MS in plasma, blood and DBS
- Cmax, tmax, AUC0-∞, AUC0-12 and t1/2 were calculated with WinNonLin

Pharmacogenetic assessments (PG)
- DNA was isolated from DBS and WB and qualified for DNA recovery
- SNP genotyping was performed for a series of allele-specific polymorphisms (CYP2D6 (*3, *4, *6), CYP2C19 (*2, *3), CYP3A4 (*1B, *2), and CYP3A5 (*3C))

Questionnaires for volunteer and staff interviews
- Subjects and phlebotomists were questioned to evaluate and compare the used sampling techniques. Input from GSK in Ware, UK was warmly welcomed herein.

Figure 1. An impression of the workflow for DBS sampling in QPS' phase I unit

Table 3. Summary statistics for PK parameters determined for WB, DBS from venous blood and DBS from finger puncture

Parameter	Mean	StDev	Median	Min	Max	Parameter	Mean	StDev	Median	Min	Max
Cmax	36.8	14.7	32.5	12.3	63.7	AUC(0-12)	79.8	28.1	82.2	45.2	132.8
(ng/mL)	29.0	8.3	27.9	15.3	42.9	(hr*ng/mL)	73.2	24.7	73.1	41.3	118.2
	23.4	7.3	24.8	10.1	34.8		53.9	18.2	52.8	32.9	93.3
Tmax	1.139	0.867	0.658	0.500	2.500	T1/2	2.59	0.77	2.49	1.24	4.17
(hr)	1.229	0.842	0.750	0.500	2.500	(hr)	4.14	1.56	3.68	2.66	7.90
	1.188	0.840	0.750	0.500	2.500		3.45	1.31	3.04	1.44	5.78
AUCinf	83.1	29.1	83.8	47.3	138.0	Kel	0.293	0.101	0.279	0.170	0.560
(hr*ng/mL)	81.0	26.7	81.8	48.9	123.7	(1/hr)	0.184	0.052	0.188	0.090	0.260
	57.6	18.7	54.2	35.7	95.9		0.232	0.098	0.228	0.120	0.480

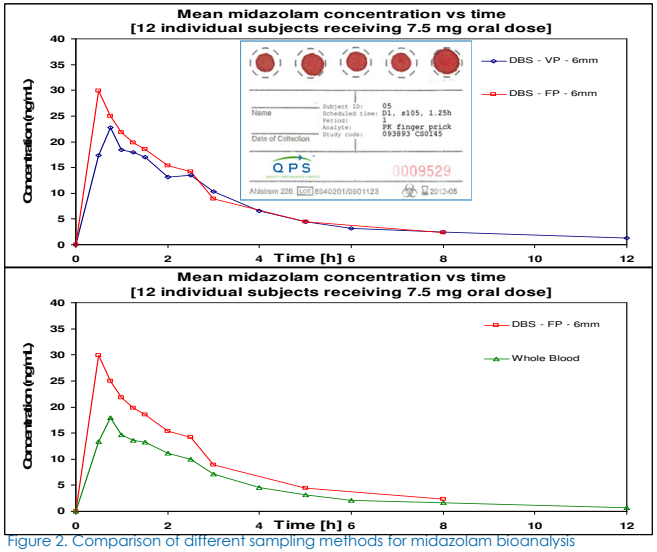

Figure 2. Comparison of different sampling methods for midazolam bioanalysis

Table 1. Blood collection volumes per collection method and per volunteer

	Frequency	Total (mL)
Safety laboratory	1 x 15 mL	15
Venipuncture for plasma	14 x2 mL	28
Venipuncture whole blood	14 x 2 mL	28
Finger puncture	10 x 0.1 mL	1.0
Total blood volume		72.0

Table 2. Results of subjects' evaluation of DBS sampling

Category	Description	Selection	N (%)
Finger Prick	Rating pain / discomfort	Mild	9 (75.0%)
		Moderate	1 (8.3%)
		None	2 (16.7%)
	Finger puncture tolerated per time point	1x	2 (16.7%)
		2x	3 (25.0%)
		3x	7 (58.3%)
	Finger puncture tolerated per day	11-15	9 (75.0%)
		6-10	3 (25.0%)
	Participate again	Yes	12 (100.0%)
Cannula	Rating pain / discomfort	Mild	5 (41.7%)
		None	7 (58.3%)
Preferred sampling		Finger prick	3 (25.0%)
		Cannula	9 (75.0%)

RESULTS

Pharmacokinetic analysis
- In figure 2 some comparisons between the sampling methods and their mean PK curves from 12 volunteers are graphically presented; a summary in Table 3 (midazolam).
- Generally, no significant differences were observed between the PK parameters after venous WB sampling and finger puncture DBS sampling, for midazolam and its 1-hydroxy metabolite.

Pharmacogenetic analysis
- For 100% of the interrogated gene alleles, WB and DBS genotyping data matched, despite slightly lower genomic DNA yield and DNA purity (A260/A280 ratios) for DBS samples.

Evaluation by subjects (study volunteers)
- Data are presented in summary in Table 2
- Generally, volunteers preferred the cannula method, but finger puncture for DBS sampling was also considered to be an acceptable sampling method, also multiple time points in 24 h.

Evaluation by (paramedic) staff
- Staff reported similar opinions on ease of use of DBS sampling versus venous sampling
- use and treatment of cards and spotting were all considered extremely easy to easy for all clinical staff involved (n= 8), of which only 1 had little prior experience

CONCLUSIONS

Conducting a clinical study with DBS sampling within our CPU and bioanalytical laboratory is feasible for phase I like studies

DBS sampling proved to be suitable for PK (including metabolite) and PG profiling

Subjects and phlebotomists did not report critical objections

In this study design, no significant differences were observed in the PK after DBS sample analysis and whole blood

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METHODS

Gd reference standard was used for preparation of calibration and validation samples. 5uL sample and 15µL Eu (IS) were simultaneously transferred by an Agilent 1200 series autosampler, mixed and injected into the Agilent 7500C series ICP-MS using continuous flow injection (FIA), see fig 2, below.

Conditions are:

- mobile phase 5mM tetrabutyl-ammonium bisulfate
- flow rate 1mL/min
- RF power 1600 watt
- ICP-MS tuned using a Gd/Eu solution (CFA, see upper part fig 2)
- Concentric nebulizer

The monitored Gd and Eu (IS) isotopes were m/z = 155, 156, 157, 158 and 160 for Gd and Eu m/z= 151 and 153, respectively.

Data acquisition was performed via the Chemstation and Analyst software for peak integration.

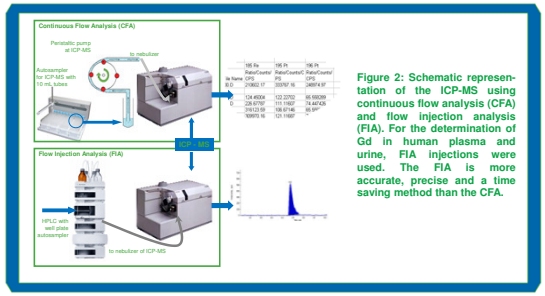


Figure 2: Schematic representation of the ICP-MS using continuous flow analysis (CFA) and flow injection analysis (FIA). For the determination of Gd in human plasma and urine, FIA injections were used. The FIA is more accurate, precise and a time saving method than the CFA.

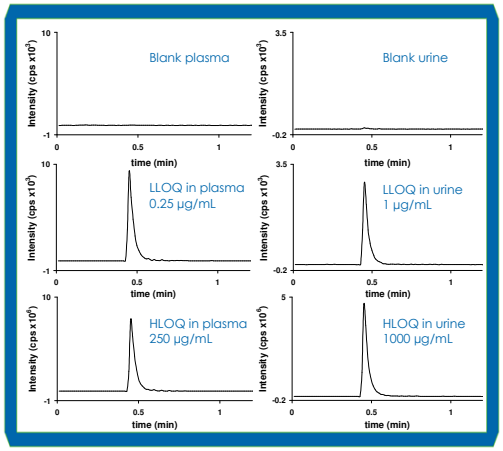
VALIDATION SET UP

For the validation of a method for the determination of total gadolinium in plasma was performed according the EMEA / FDA guidelines.

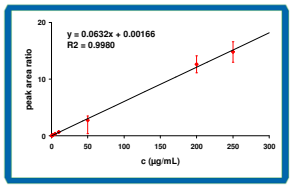
Validation design	**3 batches in 5-fold (precision and accuracy)**
Precision (CV%)	**≤5% for LLOQ, QClow, QCmed and QChigh (n=15).**
Accuracy (bias%)	**≤5% for LLOQ, QClow, QCmed and QChigh (n=15).**

Calibration curves, response function, selectivity, stability, dilution of samples, carry-over and matrix effect: validated according to standard criteria

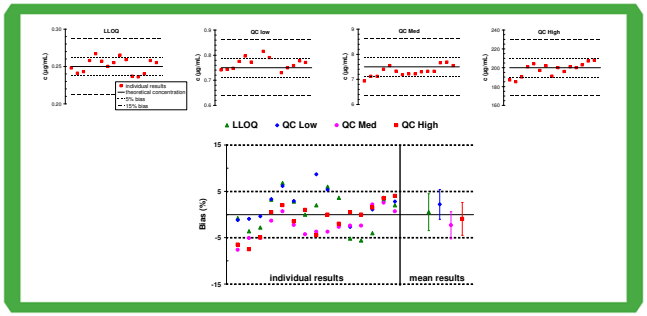
RESULTS



The validation results in **plasma**:
Selectivity: the method was selective for the determination of Gd.
Calibration curves: linear model with weighting factor 1/xx.
The correlation coefficients were ≥ 0.9972.



Precision and accuracy: the CV% of the Gd method was ≤4.0% at all concentrations. The bias was between -2.3% and 0.5% (see figure 4 for individual levels).



Freezing and thawing stability: samples could be 4 freezing and thawing cycles.
Stability of sample extracts at 2-8 °C: stable at RT for 6 days.
Bench-top stability: samples could be stored at RT for 24 hours.
Long-term stability: samples could be stored at -20 °C and -70 °C for 129 and 79 days. Long-term stability is still under investigation.
Dilution: samples could be diluted 10-fold with human plasma.
Carry over: no carry over was observed during the assay.
Matrix effect: the response in study samples will not be matrix effects.

The validation results in **urine**: still under investigation.

CONCLUSIONS

An assay was developed for the determination of Gd in human plasma and urine samples with precision and accuracy < 5% instead of the normally <15% criterion. These high precision and accuracy make it exceedingly suitable for application of Gd in contrast agents.

The excellent precision and accuracy was obtained by optimized ICP-MS parameters and conditions and by adding the internal standard through the Agilent autosampler instead of pipeting by hand.

Simultaneously determination of free and complex bound Gd by LC-ICP-MS is currently under investigation.

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