Incorporation of Microsampling Techniques in Bioanalytical Assays

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INTRODUCTION

Current pharmacokinetic (PK) and toxicological studies pose an increasing demand on the volume of blood available from study animals. Splitting collected blood sample between hematology, clinical chemistry, toxicokinetics and possibly immunological analysis can be extremely challenging, especially when the study involves small rodents. A significant number of additional animals as satellite groups are commonly incorporated into studies to provide sufficient blood volume to support all the necessary tests. Despite the benefits from ethical perspective, using satellite groups has scientific limitations, such as increased complexity of PK data interpretation caused by composite and variable profiles. As a result, microsampling techniques, such as Dried Blood Spot (DBS) and Capillary Microsampling (CMS), have been introduced into toxicological and bioanalytical studies.

Advantages of DBS:

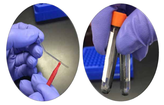
- Significant reduction in blood sample volume collected
- Simplified collection/storage/shipping
- Safe and lower cost

Advantages of CMS (Capillary Microsampling) plasma:

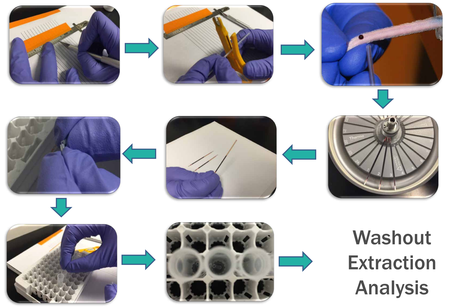
- Collect one drop of blood in a capillary tube (with anticoagulant)
- Separate plasma from blood content by centrifugation
- Transfer plasma 'end to end' into a new capillary with exact volume
- Store the capillary in a sample tube at appropriate condition
- Wash out plasma samples with proper reagent
- Perform extraction and bioanalysis
- Washed-out samples are stored properly for further use

Limitations in bioanalytical assays using conventional capillary microsampling technique:

- One aliquot of assay sample is available
- Accurate 'end to end' capillary sample transfer
- Potential over-dilution during wash-out step
- Potential risks for contamination or damage
- One chance shot



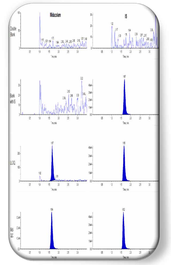
Novel & Unique Capillary Microsampling Method by Frontage



To help address the limitations of conventional CMS, a novel method of using pre-scored plastic capillaries was developed by Frontage.

Advantages out of the Frontage method:

- Use plastic tube vs. glass tube
- Multiple sample available for initial sample analysis, reanalysis, and ISR test
- Avoid issues related to sample timing; 5 min, 15 min, etc.
- Avoid the sample dilutions on site vs. other CMS method with sample dilution
- Avoid possible BQL samples due to dilution
- Application in the pediatric studies: (Overall ultimate sensitivity of method given the small plasma volumes being used)



RESULT

Determination of Midazolam in Human K2EDTA Plasma Using Capillaries

	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
Nominal concentration (ng/mL)	1	2	10	50	100	400	800	1000
Calculated concentration (ng/mL)	0.991	2.04	9.82	49.5	99	402	807	1010
%Bias	-0.7	1.8	-1.8	-0.9	-1	0.4	0.8	1.2
%CV	3.5	6.7	2	0.7	0.8	1.6	1.1	1.6
Overall R^2	0.9996							
Intra-runs	3							

	Low QC			Mid QC			High QC		
	Pipet QC	3.3 µL Capillary	10 µL Capillary	Pipet QC	3.3 µL Capillary	10 µL Capillary	Pipet QC	3.3 µL Capillary	10 µL Capillary
Replicate	18	18	18	18	18	18	18	18	18
Nominal Concentration (ng/mL)	3	3	3	75	75	75	750	750	750
Calculated Concentration (ng/mL)	2.88	2.89	2.88	78.7	72.9	71.9	743	726	732
%Bias	-4.1	-3.7	-4	4.9	-2.8	-4.1	-1	-3.2	-2.4
%CV	5	5.9	6.9	1.9	2.2	3	2.9	2.4	1.9

	Empty 5mm cuts	5mm cuts filled with H_2O
Replicate	36 (6 × 6)	36 (6 × 6)
Mean	11.99 mg	15.28 mg
%CV	2.5	2.4
Average volume of a 5 mm capillary cut	3.3 µL	

Extraction Efficiency (%)		
	3.3 µL capillary	10 µL capillary
QC Low	96.0	96.3
QC Mid	98.2	96.2
QC High	101.1	98.4
Accuracy of 50-fold diluted QC (%)	99.3	98.8

- Calibrations curves for midazolam quantitation by LC-MS/MS using Frontage-CMS showed excellent precision & accuracy over 3 runs.
- Quality control samples at low, mid and high concentration from Frontage CMS met typical bioanalytical acceptance criteria and were comparable to conventional CMS techniques.
- Extraction efficiency for Frontage-CMS was demonstrated and comparable to conventional CMS.

Frontage expertise with DBS:

- Developed and validate DBS-LC/MS/MS methods
- Supported GLP /clinical studies
- Developed standardized DBS collection, processing, storage, shipping procedure

CONCLUSIONS

Capillary microsampling (CMS) techniques for bioanalytical assays offers advantages scientifically and ethically. Frontage Laboratories has developed and validated 19 CMS-LC-MS/MS methods and successfully used these techniques in routine practices. In addition, Frontage developed a novel and unique method using pre-scored capillaries, which overcomes the limitations associated with conventional CMS approach. Frontage Laboratories has also successfully developed and fully validated 11 DBS-LC-MS/MS methods for structurally diverse compounds. Microsampling techniques are routinely used at Frontage to support GLP studies with good ISR performance. We have also supported clinical studies with both plasma and DBS samples and we are able to use the data from to generate accurate PK profiling with carefully evaluated partitioning factors.

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