

# LC/MS/MS Assay Method for Quantitation of Drug in Dried Blood Spot (DBS)

## Introduction

Traditional PK studies usually require a significant number of test animals in a composite sampling design to provide adequate sample volumes and time points for measurement. The Dried Blood Spot (DBS) technique has recently gained popularity owing to the small sample volumes it requires. This enables the use of fewer test animals in a serial sampling design to produce PK data with higher quality at a lower cost. In view of this increased utilization of DBS for PK studies, BRI has successfully implemented the use of DBS assay to improve quality of mouse PK data and lowered the cost of PK studies.

## Method

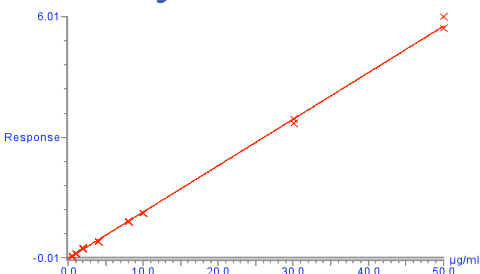
### Extraction

Card type: Whatman DMPK  
Punch Diameter: 6 mm  
Extraction Solvent:  
1%TCA in 20%ACN

### LC/MS/MS

Mobile Phases:  
0.1% formic acid in water / ACN  
Column: Synergi Hydro-RP80A  
Waters Micromass Quattro® Micro  
MS/MS ESI Positive Ion Mode

## Assay Performance



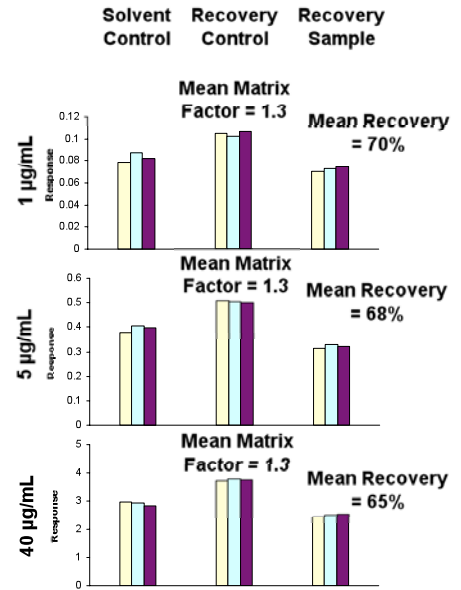
**Fig. 4** Calibration Curve of BRI-101 in DBS  
Type: Linear, Origin: Exclude, Weighting: 1/x  
 $y = 0.115708x - 0.0112596$ ,  $R^2 = 0.998592$

Calibration Level (µg/mL)	Accuracy (%Bias)	
	Curve 1	Curve 2
0.50	-9.8	1.3
1.0	-0.2	9.9
2.0	5.5	15
4.0	-10	-7.4
8.0	1.1	-0.8
10	-2.0	-2.8
30	-3.0	0.0
50	-0.9	4.1

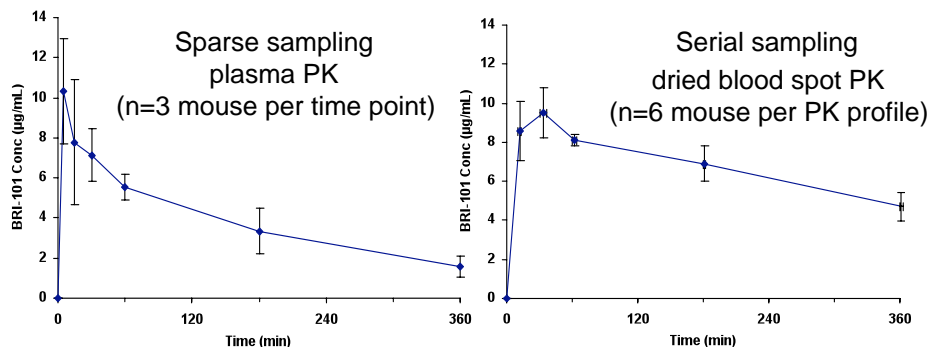
  

QC Level (µg/mL)	Intra-Assay Accuracy Precision	
	(%Bias)	(%CV)
1.0	5.4	4.7
5.0	2.3	5.7
40	-4.3	5.1

**Table 1.** Calibration and QC results for LC/MS/MS quantitation of BRI-101 in DBS indicating accuracy and precision within  $\pm 15\%$ .



**Fig. 2** Recovery results for LC/MS/MS quantitation of BRI-101 in DBS showing matrix effect at 1.3 and recovery at 65% - 70%.



**Fig. 3** Time-courses of BRI-101 in plasma from sparse sampling (left) and DBS from serial sampling (right) of six mice following single IP doses. LC/MS/MS method was successfully implemented for measurement of *in vivo* samples.

## Conclusion

- An LC/MS/MS assay has been implemented for the quantitation of BRI-101 in mouse Dried Blood Spot (DBS) to provide mouse PK data
- The DBS serial sampling approach produces PK data with higher quality at a lower cost.

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