

# Evaluation of the Dried Blood Spot technique for the quantification of diltiazem in human blood



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## Background

There is renewed interest in the development of the Dried Blood Spot technique (DBS) for determining drug levels in blood samples. The benefits of this sampling technique for pre-clinical and clinical bioanalytical assays have been recognised by BASi.

- Refinement - elimination/reduction of rodent warming
- Reduction – reduced volumes enables serial sampling from one rodent reducing animal numbers and eases sampling in paediatric studies
- Reduced costs – animal numbers, test substance, simplified procedures (collection and laboratory), shipping and storage

## Objective

- To evaluate the use of DBS to determine diltiazem in human blood.

## Methodology

- The concentration of diltiazem in excised paper discs taken from dried blood spots was determined incorporating a deuterated internal standard and analysed by LC-MS/MS. This approach was adapted from a validated BASi UK method for the quantitation of diltiazem in human plasma.
- Multiple spots/sample were made each of 40 µL/spot. Up to three discs were taken manually from individual blood spots and two types of paper with different coatings were compared (example paper shown in figure).



## Assessments

- Homogeneity and selectivity of DBS was assessed using 6 lots of individual blood applied at 40 µL/spot. One disc was taken from each DBS calibration standard (range 1.0 – 500 ng/mL) and 3 discs taken from each QC DBS. Acetonitrile:water (10:90 v/v) containing internal standard was added and the discs extracted (Table 1). The effect of excising only one disc from the centre of each QC DBS was also evaluated (Table 2).
- The effect of paper type on analyte determination was evaluated using Whatman FTA Elute and ID Biological Systems 226 collection cards.
- In an effort to increase the percentage of blood taken with each disc, the amount of blood/spot was reduced to 15 µL and a single disc excised (Table 3).

## Results

- Overall results passed the BASi accuracy and precision acceptance criteria (Table 1). Although, some individual results were inconsistent, possibly due to the non uniform nature of the DBS.

Table 1: 40 µL/spot evaluation – triplicate discs from One DBS

	Concentration (ng/mL)	1.00	3.00	150	400
Run 1					
	Intra-run Mean	1.07	2.85	140	398
	Intra-run SD	0.143	0.254	11.5	34.6
	Intra-run %CV	13.4	8.9	8.2	8.7
	Intra-run %Bias	7.0	-5.0	-6.7	-0.5
	n	6	6	6	6
Run 2					
	Intra-run Mean	1.03	3.02	150	423
	Intra-run SD	0.0709	0.264	8.13	43.5
	Intra-run %CV	6.9	8.7	5.4	10.3
	Intra-run %Bias	3.0	0.7	0.0	5.8
	n	6	5	6	6
Run 3					
	Intra-run Mean	1.03	3.16	133	389
	Intra-run SD	0.162	0.25	12.5	24.2
	Intra-run %CV	15.7	7.9	9.4	6.2
	Intra-run %Bias	3.0	5.3	-11.3	-2.8
	n	6	6	6	6
<b>Mean Concentration (ng/mL)</b>		<b>1.05</b>	<b>3.01</b>	<b>141</b>	<b>403</b>
	<b>Inter-run SD</b>	<b>0.125</b>	<b>0.274</b>	<b>12.7</b>	<b>36</b>
	<b>Inter-run %CV</b>	<b>11.9</b>	<b>9.1</b>	<b>9.0</b>	<b>8.9</b>
	<b>Inter-run %Bias</b>	<b>5.0</b>	<b>0.3</b>	<b>-6.0</b>	<b>0.8</b>
	<b>n</b>	<b>18</b>	<b>17</b>	<b>18</b>	<b>18</b>

- The ID Biological Systems 226 collection cards were not able to retain the blood from the highly aqueous solution used in the extraction. In consequence, the resulting extractions appeared red in colour producing a 'dirty' sample, thus unsuitable for analysis.
- The overall results from the single excised disc experiment again passed the BASi accuracy and precision acceptance criteria (Table 2). However, again some individual results were inconsistent and further investigation is required.
- Results indicate that increasing the percentage of sample taken for analysis increases the accuracy of the DBS technique (Table 3). Further experimentation will be done to confirm this finding.

Table 2: 40 µL/spot evaluation – Individual disc from one DBS

	Concentration (ng/mL)	1.00	3.00	150	400
Run 1					
	Intra-run Mean	1.03	2.76	144	448
	Intra-run SD	0.118	0.284	9.54	34.5
	Intra-run %CV	11.5	10.3	6.6	7.7
	Intra-run %Bias	3.0	-8.0	-4.0	12.0
	n	6	6	6	6
Run 2					
	Intra-run Mean	1.15	2.80	145	441
	Intra-run SD	0.108	0.275	9.00	45.4
	Intra-run %CV	9.4	9.8	6.2	10.3
	Intra-run %Bias	15.0	-6.7	-3.3	10.3
	n	6	6	6	6
Run 3					
	Intra-run Mean	0.966	3.07	145	458
	Intra-run SD	0.0918	0.190	8.42	22.4
	Intra-run %CV	9.5	6.2	5.8	4.9
	Intra-run %Bias	-3.4	2.3	-3.3	14.5
	n	6	6	6	6
<b>Mean Concentration (ng/mL)</b>		<b>1.05</b>	<b>2.88</b>	<b>145</b>	<b>449</b>
	<b>Inter-run SD</b>	<b>0.126</b>	<b>0.277</b>	<b>8.46</b>	<b>34.0</b>
	<b>Inter-run %CV</b>	<b>12.0</b>	<b>9.6</b>	<b>5.8</b>	<b>7.6</b>
	<b>Inter-run %Bias</b>	<b>5.0</b>	<b>-4.0</b>	<b>-3.3</b>	<b>12.3</b>
	<b>n</b>	<b>18</b>	<b>18</b>	<b>18</b>	<b>18</b>

Table 3: 15 µL/spot evaluation – 1 disc per spot

	Concentration (ng/mL)	1.00	3.00	150	400
	Intra-run Mean	1.00	2.83	144	362
	Intra-run SD	0.0741	0.168	13.7	20.9
	Intra-run %CV	7.4	5.9	9.5	5.8
	Intra-run %Bias	0.0	-5.7	-4.0	-9.5
	n	6	6	6	6

## Conclusion

The DBS technique was used successfully to determine the concentration of diltiazem in human blood samples. The reliability of the technique for 40 µL blood spots will require further investigation but increasing the percentage of sample taken for analysis, by only spotting 15 µL of blood, increased its precision.

## Future research

BASi intends to follow up this work investigating alternative papers e.g. ID Biological Systems 226. A further evaluation of the DBS technique will be performed to determine gemcitabine in whole blood and plasma, normally a difficult process due to rapid gemcitabine deamination.