



Blood Plasma Partition Assay

The Physiological Distribution/Elimination model requires blood plasma partitioning (BPP) and hematocrit (HCT) data collected in the blood plasma partition assay as input parameters.

BPP is equivalent to the blood-plasma concentration ratio (C_b/C_p). Additionally, the D/E model requires that the BPP and HCT data satisfy the equation:

$$\text{BPP} + \text{HCT} > 1$$

BPP data from the 2mM concentration is suggested as input into the model. The BPP data from the 0.4mM and 10mM concentrations are alternate inputs.

In the case where the $\text{BPP} + \text{HCT} \leq 1$, the BPP assay should be repeated in a matrix with higher red blood cell content and/or at the 10mM concentration. The 0.4mM is useful for cases when the 2mM concentration is difficult to achieve, as may be the case during the early drug discovery phase when there is low compound availability.

Blood Plasma Partition Assay

Goal

To measure the drug or compound concentration ratio between blood and plasma.

Set-up

MATERIALS

- Blood (ca 65 ml): At least 3 donors in pool (fresh or 2-3 days old purchased from blood bank). purchased the blood from San Diego Blood Bank.
- ca 46 ml: blood dosing solution
- ca 5 ml: counter dilution for plasma sample
- ca 14 ml (2 x 7 ml in PP tube): ca 5 ml plasma for counter dilution of blood sample
- Blood, plasma, and blood dosing solution will be kept on ice before starting incubation
- 96-shallow and 96-deep well plates

- Heparinized microhematocrit (HCT) capillary tube: Fisher Scientific (Cat 22-362-566)

SOLUTIONS

- Stock solution: 10 mM in DMSO
- Working solutions: BLK, 0.04, 0.2, and 1 mM 10% DMSO in saline
- Dosing solutions in blood: BLK, 0.4, 2, and 10 μ M in blood (0.1% DMSO)
- Blood, plasma, heparin sodium injection (1000 IU/ml, Elkins-Sinn product #2440), saline, and 10% DMSO in saline
- Extraction solution: water:MeCN=1:1 including 0.1% ZnSO₄
- Washing solution: MeOH:water=1:1

Table 60. Working Solutions Format in 96 Shallow Well and Preparation (40 μ L)

	Col 1	Col 2	Col 3	Col 4								
Comp 1	BLK	0.04 mM	0.2 mM	1 mM								
Comp 2	BLK	0.04 mM	0.2 mM	1 mM								
Comp 3	BLK	0.04 mM	0.2 mM	1 mM								
Comp 4	BLK	0.04 mM	0.2 mM	1 mM								
Comp 5	BLK	0.04 mM	0.2 mM	1 mM								
Comp 6	BLK	0.04 mM	0.2 mM	1 mM								
Comp 7	BLK	0.04 mM	0.2 mM	1 mM								
Comp 8	BLK	0.04 mM	0.2 mM	1 mM								

1. Transfer 45 μ L saline in Col 4.
2. Transfer 40 μ L 10% DMSO in saline in Col 1, Col 2, and Col 3.
3. Transfer 5 μ L 10 mM DMSO stock into Col 4 and mix it by repetitive pipetting to prepare 1 mM.
4. Transfer 10 μ L of Col 4 soln to Col 3, and mix it by repetitive pipetting to prepare 0.2 mM.

5. Transfer 10 μL of Col 3 soln to Col 2, and mix it by repetitive pipetting to prepare 0.04 mM.

Protocol summary

- Human blood: At least 3 donors in pool (fresh or 2-3 days old purchased from blood bank)
- Heparinized blood (25 IU/ml)
- 96 well format using shallow well plate
- Concentration: BLK, 0.4, 2, and 10 μM in blood (w 0.1% DMSO)/ n=3
- 200 μL blood dosing solutions in blood sample plate and plasma sample plate
- Incubation: 1 hr in a 37°C humidified incubator/ 50 rpm
- Blood sample: 40 μL blood from the blood sample plate
- Plasma sample: 40 μL plasma from the plasma sample plate centrifuged at 2000 g/ 4 °C/ 5 min
- 40 μL counter part added to sample plates (ex, 40 μL blank blood added to plasma sample)
- Sample extraction: treated with 160 μL extraction solution (water:MeCN=1:1 including 0.1% ZnSO_4)
- Sample analysis: LC/UV or LC/MS
- Output: C_b/C_p (blood-plasma concentration ratio), C_b : blood conc and C_p : plasma conc
- Hematocrit measurement: Centrifugation of microhematocrit capillary tube at 2000 g/ 4°C/ 30 min
- Amount required: Approximately 10 μL of 10 mM stock solution in DMSO
- Throughput: 8-32 compounds/day/1 FTE, but general procedures for 8 compounds are described here

Experimental procedures

Table 61. Blood Dosing Solutions Format in 96-Deep Well and Preparation (1.4 ml)

	Col 1	Col 2	Col 3	Col 4								
Comp 1	BLK	0.4 μ M	2 μ M	10 μ M								
Comp 2	BLK	0.4 μ M	2 μ M	10 μ M								
Comp 3	BLK	0.4 μ M	2 μ M	10 μ M								
Comp 4	BLK	0.4 μ M	2 μ M	10 μ M								
Comp 5	BLK	0.4 μ M	2 μ M	10 μ M								
Comp 6	BLK	0.4 μ M	2 μ M	10 μ M								
Comp 7	BLK	0.4 μ M	2 μ M	10 μ M								
Comp 8	BLK	0.4 μ M	2 μ M	10 μ M								

1. Transfer 14 μ L working solutions to corresponding blood dosing solution 96-deep well plate using an 8 channel pipette/ keep it on ice.
2. Prepare a tube with heparin (1.2 ml Heparin solution in a 50 ml conical tube).
3. GENTLY pour out blood (about 16 ml from each pint/Pool three pints, then about total 48 ml blood/tube) to the heparin tube, and gently mix it.
4. Transfer 1400 μ L blood and mix it by rotating tubes (for 5–10 min). Note: **DO NOT VORTEX**.

Table 62. Incubation Plate Format in 96 Shallow Well and Preparation (200 μ L)

	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10	Col 11	Col 12
Comp 1	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
Comp 2	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
Comp 3	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
Comp 4	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
Comp 5	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
Comp 6	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
Comp 7	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
Comp 8	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M

1. Transfer 200 μ L blood dosing solutions to the incubation plate (2 plates for blood sample and plasma sample) using 8 channel pipette.

2. Incubate the plates for 1 hr in a 37°C humidified incubator/ at 50 rpm.
3. During incubation, prepare blk plasma by centrifuging blood at 2000g for 30 min.
4. During incubation, fill 40 µL blood into HCT capillary (at least 4) and centrifuge at 2000g for 30 min to measure HCT.

Table 63. Extraction plate format in 96 shallow well and preparation:
160 µL Extraction buffer + 40 µL sample + 40 µL counter part

	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10	Col 11	Col 12
Comp 1	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM
Comp 2	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM
Comp 3	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM
Comp 4	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM
Comp 5	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM
Comp 6	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM
Comp 7	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM
Comp 8	BLK	BLK	BLK	0.4 µM	0.4 µM	0.4 µM	2 µM	2 µM	2 µM	10 µM	10 µM	10 µM

1. Transfer 160 µL Extraction solution (water:MeCN=1:1 including 0.1% ZnSO₄) to the extraction plate (2 plates for blood sample and plasma sample). Keep extraction plates on ice.
2. One hour after incubation, centrifuge plasma incubation plate for 5 min at 2000g/4°C. Transfer 40 µL plasma sample to the corresponding plasma extraction plate. Mix it by repetitive pipetting.
3. One hour after incubation, mix blood samples of blood incubation plate by repetitive pipetting, and transfer 40 µL blood sample to the corresponding blood extraction plate. Mix it by repetitive pipetting.
4. Plasma extraction plate: Add 40 µL blank blood. Mix it by repetitive pipetting.
5. Blood sample plate: Add 40 µL blank plasma. Mix it by repetitive pipetting.
6. Store blood and plasma extraction plates at -80°C/ 1 hr, and centrifuge them at 2000 g/ 4°C/ 1 hr.

Table 64. Bioanalytical Plate Format in 96-Shallow Well and Preparation (ex., Compound 1)

	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10	Col 11	Col 12
	Wash			Wash			Wash			Wash		
Blood	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M
	Wash			Wash			Wash			Wash		
plasma	BLK	BLK	BLK	0.4 μ M	0.4 μ M	0.4 μ M	2 μ M	2 μ M	2 μ M	10 μ M	10 μ M	10 μ M

1. Transfer 100 μ L washing solution (MeOH:water=1:1) to row 1 and 3 as indicated.
2. Transfer 80-90 μ L supernatant of blood extraction plate to row 2.
3. Transfer 80-90 μ L supernatant of plasma extraction plate to row 4.
4. Sample injection: wash-blood blank (n=3)-wash-plasma blank (n=3).
5. Next sample injection: sequentially from 0.4 μ M to 10 μ M.

OUTPUT DATA

Output data: C_b/C_p (Blood-plasma concentration ratio)

1. Blood conc (C_b): Blood area-BLK area
2. Plasma conc (C_p): Plasma area-BLK area
3. C_b/C_p = Blood conc/ Plasma conc

Table 65. Example Data of C_b/C_p

	0.04 μ M	SD	2 μ M	SD	10 μ M	SD	Hepatocrit
Atenolol	0.914	0.08	1.029	0.04	0.972	0.02	Not determined
Phenylbutazone	0.433	0.01	0.461	0.24	0.504	0.08	0.4
Propranolol	1.41	0.03	1.417	0.1	1.512	0.05	0.415

Example Blood Plasma Partition Template

See Figure 42 for an example of the Blood Plasma Partition template and results graph.

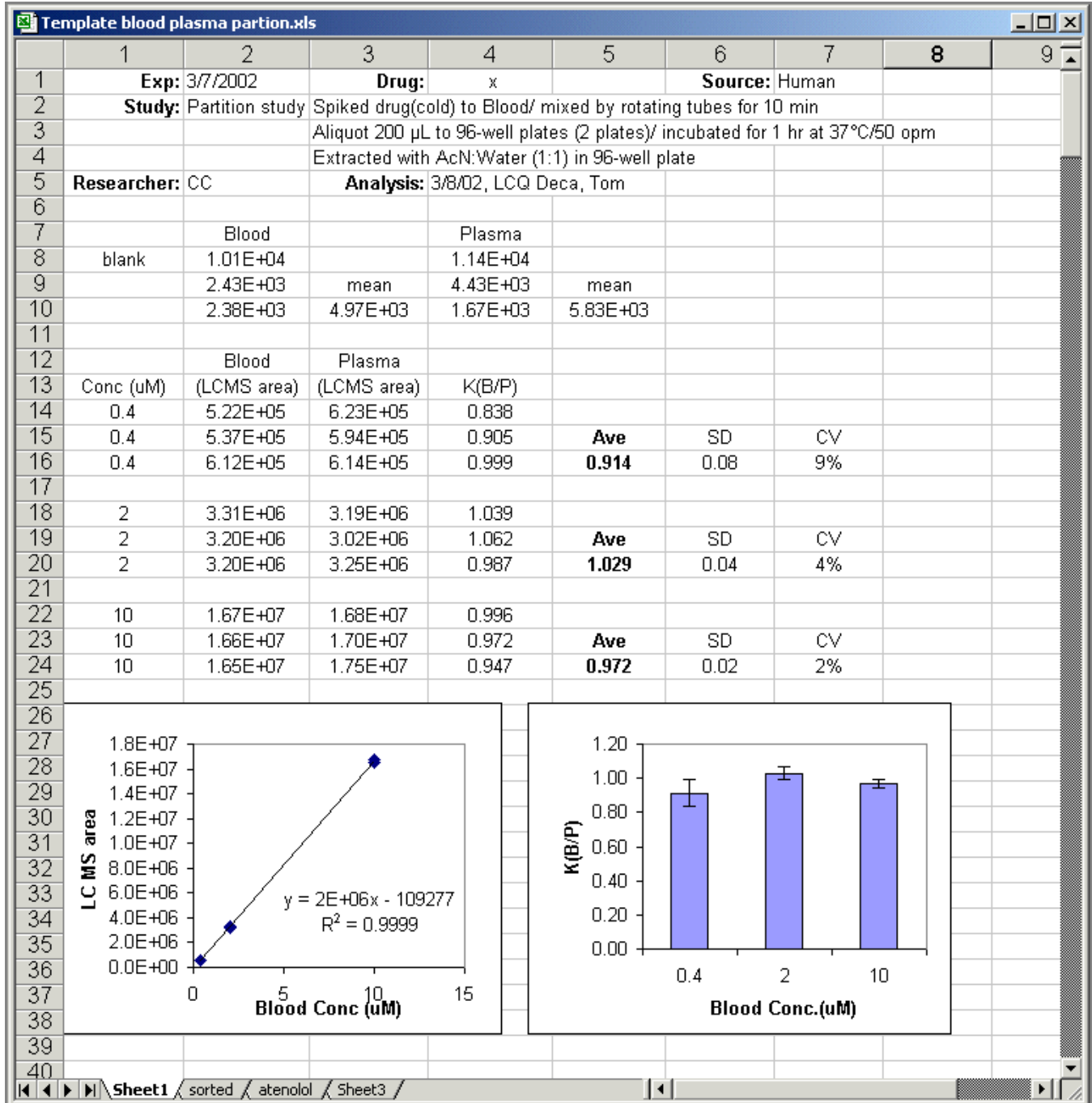


Figure 42. Blood Plasma Partition Template