

Short Communication

Supplemental Materials

**Applying Stable Isotope Labeled Amino Acids in Micropatterned Hepatocyte Co-Culture
to Determine the Degradation Rate Constant for CYP3A4**

Supplemental Table 1. Donor information for hepatocytes used for HepatoPac cultures.

Donor	3121A	3121B	4202	4297
Sex	Female	Female	Female	Female
Age	52	48	39	54
Height/Weight	65" / 48.2 kg	66" / 59 kg	63" / 90 kg	
Cause of Death	Anoxia, 2nd to Cardiac Arrest	Trauma	Anoxia, 2nd to Cardiac Arrest	Stroke
Ethnicity	Caucasian	Polynesian	Caucasian	Caucasian
Medical History	COPD. No EtOH; Tobacco: 2ppd x 35 years, Drugs: Marijuana Daily	Diverticulitis w/ Surgery. EtOH: Mild	HTN x 3yrs - non-compliant. EtOH: Occasionally; Tobacco: 1/2ppd x 10 years; Drugs: abused Rx narcotics, smoked marijuana.	No drug or tobacco use, 1 glass of wine per day

Supplemental Table 2. Summary of tryptic peptides and their selected reaction-monitoring parameters used for LC-MS/MS protein measurements. Stable isotopes were at the terminal lysine or arginine for stable isotope labeled (SIL) and internal standard (IS) peptides.

P450	Peptide	Q1 (no SIL/SIL)	Q3 (no SIL/SIL)	Observed Retention time (min)	DP	CE
	SLLSPTFTSGK	569/572 ⁺⁺	824/830 ⁺ (y8)	18.4	73	29
	EVTNFLR	439/442 ⁺⁺	650/656 ⁺ (y5)	14.3	63	25
CYP3A4	EVTNFLR (IS)	444 ⁺⁺	660 ⁺ (y5)			
	DVEINGMFIPK	631/634 ⁺⁺	806/812 ⁺ (y7)	25.0	77	32
	YWTEPEK	476/479 ⁺⁺	603/609 ⁺ (y5)	9.2	66	26

+, ++, +++ indicates singly, doubly, and triply-charged ions, respectively.

Supplemental Table 3. CYP3A4 stability data (mRNA, protein expression, and enzymatic activity) for four HepatoPac donors for Days 10-13 of SILAC culture.

CYP3A4 mRNA (GAPDH normalized)				
Day	3121A	3121B	4202	4297
10	0.71±0.03	0.29±0.02		
11	0.53±0.02	0.30±0.03		
12	0.81±0.04	0.52±0.03		
13	0.69±0.05	0.49±0.08		
CYP3A4 protein (fmol per 10 ⁶ cells)				
Day	3121A	3121B	4202	4297
10	510±256	290±51	347±23	183±55
11	607±233	566±102	446±137	362±54
12	909±237	846±197	797±234	594±105
13	787±98	801±183	490±76	465±66
CYP3A4 activity (pmol 6β-hydroxytestosterone mg ⁻¹ min ⁻¹)				
Day	3121A	3121B	4202	4297
10	4406±445	3328±322	3217±293	2380±289
11	5380±721	4479±440	3717±334	2988±217
12	5100±541	4674±604	4340±367	4161±284
13	4961±509	4133±662	5089±273	4368±275

Data are reported as mean±standard deviation. mRNA was determined on n=3 pooled cultures (4 wells per pool) for each day following hepatocyte collection. Protein was determined on n=3 pooled cultures (4 wells per pool) for each day following hepatocyte collection. Activity was determined on n=12 wells for each day prior to hepatocyte collection.

Supplemental Table 4. Calculated $t_{1/2}$ (h) and k_{deg} (h^{-1}) values from individual donors for CYP3A4.

P450	Peptide	Donor	Slope	k_{deg}			$t_{1/2}$		
				(h^{-1})	mean	SD	(h)	mean	SD
CYP3A4	SLLSPTFTSGK	3121A	-0.5410	0.0225	0.023	0.002	30.7	29.6	1.9
		3121B	-0.6174	0.0257			26.9		
		4202	-0.5349	0.0223			31.1		
		4297	-0.5621	0.0234			29.6		
	EVTNFLR	3121A	-0.7411	0.0309	0.033	0.005	22.4	21.0	2.6
		3121B	-0.7566	0.0315			22.0		
		4202	-0.7381	0.0308			22.5		
		4297	-0.9685	0.0404			17.2		
	DVEINGMFIPK	3121A	-0.5653	0.0236	0.023	0.001	29.4	29.6	1.0
		3121B	-0.5892	0.0246			28.2		
		4202	-0.5427	0.0226			30.7		
		4297	-0.5534	0.0231			30.1		
	YWTEPEK	3121A	-0.5588	0.0233	0.023	0.001	29.8	29.8	0.8
		3121B	-0.5778	0.0241			28.8		
		4202	-0.5562	0.0232			29.9		
		4297	-0.5424	0.0226			30.7		
Mean, all CYP3A4 peptides				0.026			27.5		
SD, all CYP3A4 peptides				0.005			4.3		
Mean, three lysine-containing CYP3A4 peptides				0.023			29.7		
SD, three lysine-containing CYP3A4 peptides				0.002			2.3		

Supplemental Figure 1. Representative SRM chromatograms showing CYP3A4 peptide, SLLSPTFTSGK, for one HepatoPac donor over Days 10-13 with SILAC. Top trace shows transitions monitored for non- $^{13}\text{C}_6$ -lysine containing peptide (m/z 569.3 (doubly charged)) and bottom trace shows transitions monitored for “heavy” $^{13}\text{C}_6$ -lysine containing peptide (m/z 572.3 (doubly charged)) for k_{deg} determination.

