

## Supplementary Information

### **Quantification of accurate composition and total abundance of homologous proteins by conserved-plus-surrogate peptide (CPSP) approach: Quantification of UDP glucuronosyltransferases in human tissues**

Deepak Ahire<sup>1</sup>, Mitesh Patel<sup>2</sup>, Sujal V. Deshmukh<sup>2</sup>, and Bhagwat Prasad<sup>1</sup>

Department of Pharmaceutical Sciences, Washington State University (WSU), Spokane, WA  
Novartis Institutes for BioMedical Research, Cambridge, MA

#### **Address Correspondence to:**

Bhagwat Prasad, Ph.D.  
Department of Pharmaceutical Sciences  
Washington State University  
Spokane, WA 99202  
Telephone: +1-(509) 358-7739  
Fax: +1-509-368-6561  
E-mail: [bhagwat.prasad@wsu.edu](mailto:bhagwat.prasad@wsu.edu)

**Standardization of UGT1A and UGT2B conserved peptides**

The stable isotope-labeled (SIL) UGT1A conserved peptide (IPQTVLWR) was originally procured from Thermo Fisher Scientific (Rockford, IL) without amino acid analysis (AAA). To standardize the SIL form of IPQTVLWR, we used an external calibration method (Bhatt et al., 2019) where the light IPQTVLWR peptide, procured with AAA from New England Peptide (Gardner, MA), was used as a calibrator. UGT2B conserved SIL peptide (VLWR) was standardized by AAA by the vendor (Vivitide, Gardner, MA). The linearity and range of the LC-MS method was verified by measuring the MS responses of the SIL peptides, IPQTVLWR and VLWR from 1.44 to 925.5 and 0.33 to 170.5 fmol/ $\mu$ L, respectively.

<b>Supplementary Table 1:</b> List of conserved and surrogate peptides used for targeted LC-MS/MS quantification of UGT proteins. Light peptides are unlabeled, whereas heavy peptides contain stably labeled ( $^{13}\text{C}$ and $^{15}\text{N}$ ) R or K						
Protein	Peptide sequence	Peptide label	Parent ion (m/z)	Product ion (m/z)	CE (eV)	Cone voltage (V)
<b>Conserved peptides</b>						
UGT1A	IPQTVLWR	Light	506.8	802.4	16	35
	IPQTVLWR	Light	506.8	674.4	16	35
	IPQTVLWR	Light	506.8	573.3	16	35
	IPQTVLWR	Heavy	511.8	812.4	16	35
	IPQTVLWR	Heavy	511.8	684.4	16	35
	IPQTVLWR	Heavy	511.8	583.4	16	35
UGT2B	VLWR	Light	287.2 (+2)	474.2	10	35
	VLWR	Light	287.2 (+2)	361.2	10	35
	VLWR	Light	287.2 (+2)	213.2	10	35
	VLWR	Heavy	292.1 (+2)	484.3	10	35
	VLWR	Heavy	292.1 (+2)	371.2	10	35
	VLWR	Heavy	292.1 (+2)	213.2	10	35
<b>Surrogate peptides</b>						
UGT1A1	DGAFYTLK	Light	457.7 (+2)	671.4	16	35
	DGAFYTLK	Light	457.7 (+2)	260.2	16	35
	DGAFYTLK	Light	457.7 (+2)	244.1	16	35
	DGAFYTLK	Heavy	461.7 (+2)	679.4	16	35
	DGAFYTLK	Heavy	461.7 (+2)	268.2	16	35
	DGAFYTLK	Heavy	461.7 (+2)	244.1	16	35
	ESFVSLGHNVFENDSFLQR	Light	742.4 (+3)	650.4	25	35
	ESFVSLGHNVFENDSFLQR	Light	742.4 (+3)	303.2	25	35
	ESFVSLGHNVFENDSFLQR	Light	742.4 (+3)	881.9	25	35
	ESFVSLGHNVFENDSFLQR	Heavy	745.7 (+3)	660.4	25	35
	ESFVSLGHNVFENDSFLQR	Heavy	745.7 (+3)	313.2	25	35
	ESFVSLGHNVFENDSFLQR	Heavy	745.7 (+3)	886.9	25	35
UGT1A3	YLSIPTVFFLR	Light	678.4 (+2)	1079.6	24	35
	YLSIPTVFFLR	Light	678.4 (+2)	879.5	24	35
	YLSIPTVFFLR	Light	678.4 (+2)	277.2	24	35
	YLSIPTVFFLR	Heavy	683.4 (+2)	1089.6	24	35
	YLSIPTVFFLR	Heavy	683.4 (+2)	889.5	24	35

	YLSIPTVFFLR	Heavy	683.4 (+2)	277.2	24	35
UGT1A4	VTLGYTQGGFFETEHLK	Light	661.7 (+2)	1016.5	22	35
	VTLGYTQGGFFETEHLK	Light	661.7 (+2)	892	22	35
	VTLGYTQGGFFETEHLK	Light	661.7 (+2)	835.4	22	35
	VTLGYTQGGFFETEHLK	Heavy	664.4 (+2)	1024.6	22	35
	VTLGYTQGGFFETEHLK	Heavy	664.4 (+2)	896	22	35
	VTLGYTQGGFFETEHLK	Heavy	664.4 (+2)	839.4	22	35
	GTQCPNPSSYIPK	Light	724.8 (+2)	791.4	30	35
	GTQCPNPSSYIPK	Light	724.8 (+2)	581.7	30	35
	GTQCPNPSSYIPK	Heavy	728.8 (+2)	799.4	30	35
	GTQCPNPSSYIPK	Heavy	840.9 (+2)	585.7	30	35
	UGT1A6	SFLTAPQTEYR	Light	656.8 (+2)	965.5	23
SFLTAPQTEYR		Light	656.8 (+2)	864.4	23	35
SFLTAPQTEYR		Light	656.8 (+2)	793.4	23	35
SFLTAPQTEYR		Heavy	661.8 (+2)	975.5	23	35
SFLTAPQTEYR		Heavy	661.8 (+2)	874.4	23	35
SFLTAPQTEYR		Heavy	661.8 (+2)	803.4	23	35
DIVEVLSDR		Light	523.3 (+2)	718.4	18	35
DIVEVLSDR		Light	523.3 (+2)	589.3	18	35
DIVEVLSDR		Heavy	528.3 (+2)	728.4	18	35
DIVEVLSDR		Heavy	528.3 (+2)	599.3	18	35
UGT1A7 & UGT1A8	YFSLPSVVFAR	Light	643.3 (+2)	775.4	23	35
	YFSLPSVVFAR	Light	643.3 (+2)	388.2	23	35
	YFSLPSVVFAR	Light	643.3 (+2)	311.1	23	35
	YFSLPSVVFAR	Heavy	648.3 (+2)	785.4	23	35
	YFSLPSVVFAR	Heavy	648.3 (+2)	393.2	23	35
	YFSLPSVVFAR	Heavy	648.3 (+2)	311.1	23	35
UGT1A7	TYSTSYTLEDQD	Light	789.8 (+2)	662.2	23	35
	TYSTSYTLEDQD	Light	789.8 (+2)	775.3	23	35
	TYSTSYTLEDQD	Light	789.8 (+2)	876.4	23	35
	TYSTSYTLEDQD	Heavy	794.8 (+2)	672.2	23	35
	TYSTSYTLEDQD	Heavy	794.8 (+2)	785.3	23	35
	TYSTSYTLEDQD	Heavy	794.8 (+2)	886.4	23	35
UGT1A8	GIACHYLEEGAQCPAPLSYVP	Light	830.0 (+3)	831.4	23	45
	GIACHYLEEGAQCPAPLSYVP	Light	830.0 (+3)	601.2	23	45
	GIACHYLEEGAQCPAPLSYVP	Light	830.0 (+3)	665.3	23	45
	GIACHYLEEGAQCPAPLSYVP	Heavy	833.4 (+3)	65.3	23	45
	GIACHYLEEGAQCPAPLSYVP	Heavy	833.4 (+3)	601.2	23	45
	GIACHYLEEGAQCPAPLSYVP	Heavy	833.4 (+3)	841.4	23	45

UGT1A9	AFAHAQWK	Light	320.2	444.2	23	35
	AFAHAQWK	Light	320.2	370.7	23	35
	AFAHAQWK	Light	320.2	335.2	23	35
	AFAHAQWK	Heavy	322.8	448.2	23	35
	AFAHAQWK	Heavy	322.8	374.7	23	35
	AFAHAQWK	Heavy	322.8	339.2	23	35
UGT1A10	YFSLPSVVFTR	Light	658.4 (+2)	1005.6	23	35
	YFSLPSVVFTR	Light	658.4 (+2)	805.5	23	35
	YFSLPSVVFTR	Light	658.4 (+2)	398.2	23	35
	YFSLPSVVFTR	Heavy	663.4 (+2)	1015.6	23	35
	YFSLPSVVFTR	Heavy	663.4 (+2)	815.5	23	35
	YFSLPSVVFTR	Heavy	663.4 (+2)	398.2	23	35
UGT2B4	FEVYPVSLTK	Light	591.8 (+2)	906.5	21	35
	FEVYPVSLTK	Light	591.8 (+2)	807.5	21	35
	FEVYPVSLTK	Light	591.8 (+2)	644.4	21	35
	FEVYPVSLTK	Heavy	595.8 (+2)	914.5	21	35
	FEVYPVSLTK	Heavy	595.8 (+2)	815.5	21	35
	FEVYPVSLTK	Heavy	595.8 (+2)	652.4	21	35
	TILDELVQR	Light	543.8 (+2)	872.5	19	35
	TILDELVQR	Light	543.8 (+2)	759.4	19	35
	TILDELVQR	Light	543.8 (+2)	644.4	19	35
	TILDELVQR	Heavy	548.8 (+2)	882.5	19	35
	TILDELVQR	Heavy	548.8 (+2)	769.4	19	35
	TILDELVQR	Heavy	548.8 (+2)	654.4	19	35
UGT2B7	ANVIASALAQIPQK	Light	712.4 (+2)	797.5	25	35
	ANVIASALAQIPQK	Light	712.4 (+2)	684.4	25	35
	ANVIASALAQIPQK	Light	712.4 (+2)	372.2	25	35
	ANVIASALAQIPQK	Heavy	716.4 (+2)	805.5	25	35
	ANVIASALAQIPQK	Heavy	716.4 (+2)	692.4	25	35
	ANVIASALAQIPQK	Heavy	716.4 (+2)	380.2	25	35
	IEIYPTSLTK	Light	582.8 (+2)	922.5	20	35
	IEIYPTSLTK	Light	582.8 (+2)	646.4	20	35
	IEIYPTSLTK	Heavy	586.8 (+2)	930.5	20	35
	IEIYPTSLTK	Heavy	586.8 (+2)	654.4	20	35
	TILDELIQR	Light	550.8 (+2)	886.5	19	35
	TILDELIQR	Light	550.8 (+2)	658.4	19	35
	TILDELIQR	Light	550.8 (+2)	416.3	19	35
	TILDELIQR	Heavy	555.8 (+2)	896.5	19	35
TILDELIQR	Heavy	555.8 (+2)	668.4	19	35	

	TILDELIQR	Heavy	555.8 (+2)	426.3	19	35
UGT2B10	TEFENIIMQLVK	Light	732.9 (+2)	731.4	25	35
	TEFENIIMQLVK	Light	732.9 (+2)	618.3	25	35
	TEFENIIMQLVK	Light	732.9 (+2)	487.3	25	35
	TEFENIIMQLVK	Heavy	736.9 (+2)	739.4	25	35
	TEFENIIMQLVK	Heavy	736.9 (+2)	626.3	25	35
	TEFENIIMQLVK	Heavy	736.9 (+2)	495.3	25	35
	TEFENIIMQLVK	Heavy	736.9 (+2)	495.3	25	35
UGT2B15	SVINDPVYK	Light	517.8 (+2)	848.5	18	35
	SVINDPVYK	Light	517.8 (+2)	735.4	18	35
	SVINDPVYK	Light	517.8 (+2)	424.7	18	35
	SVINDPVYK	Heavy	521.8 (+2)	856.5	18	35
	SVINDPVYK	Heavy	521.8 (+2)	743.4	18	35
	SVINDPVYK	Heavy	521.8 (+2)	428.7	18	35
	NYLEDSLLK	Light	547.8 (+2)	817.5	19	35
	NYLEDSLLK	Light	547.8 (+2)	704.4	19	35
	NYLEDSLLK	Light	547.8 (+2)	278.1	19	35
	NYLEDSLLK	Heavy	551.8 (+2)	825.5	19	35
	NYLEDSLLK	Heavy	551.8 (+2)	712.4	19	35
	NYLEDSLLK	Heavy	551.8 (+2)	278.1	19	35
	UGT2B17	FSVGYTVEK	Light	515.3 (+2)	882.5	18
FSVGYTVEK		Light	515.3 (+2)	795.4	18	35
FSVGYTVEK		Light	515.3 (+2)	696.4	18	35
FSVGYTVEK		Heavy	519.3 (+2)	890.5	18	35
FSVGYTVEK		Heavy	519.3 (+2)	803.4	18	35
FSVGYTVEK		Heavy	519.3 (+2)	704.4	18	35
SVINDPIYK		Light	524.8 (+2)	862.5	18	35
SVINDPIYK		Light	524.8 (+2)	749.4	18	35
SVINDPIYK		Light	524.8 (+2)	431.7	18	35
SVINDPIYK		Heavy	528.8 (+2)	870.5	18	35
SVINDPIYK		Heavy	528.8 (+2)	757.4	18	35
SVINDPIYK		Heavy	528.8 (+2)	435.7	18	35

<b>Supplementary Table 2:</b> Chromatographic conditions to separate UGT surrogate and conserved peptides			
Guard column: Acquity HSS T3 column (100Å, 1.8 µm, 2.1mm * 5 mm)			
Acquity UPLC HSS T3 column (100Å, 1.8 µm, 1 mm * 100 mm)			
Injection volume: 1 µL			
<b>LC gradient program</b>			
Time (min)	Flow Rate (µL)	A (Water with 0.1% formic acid, %)	B (Acetonitrile with 0.1% formic acid, %)
0	50	97	3
4	50	97	3
8	50	87	13
18	50	70	30
20.5	50	65	35
21.1	50	40	60
23.1	50	20	80
23.2	50	97	3
27	50	97	3

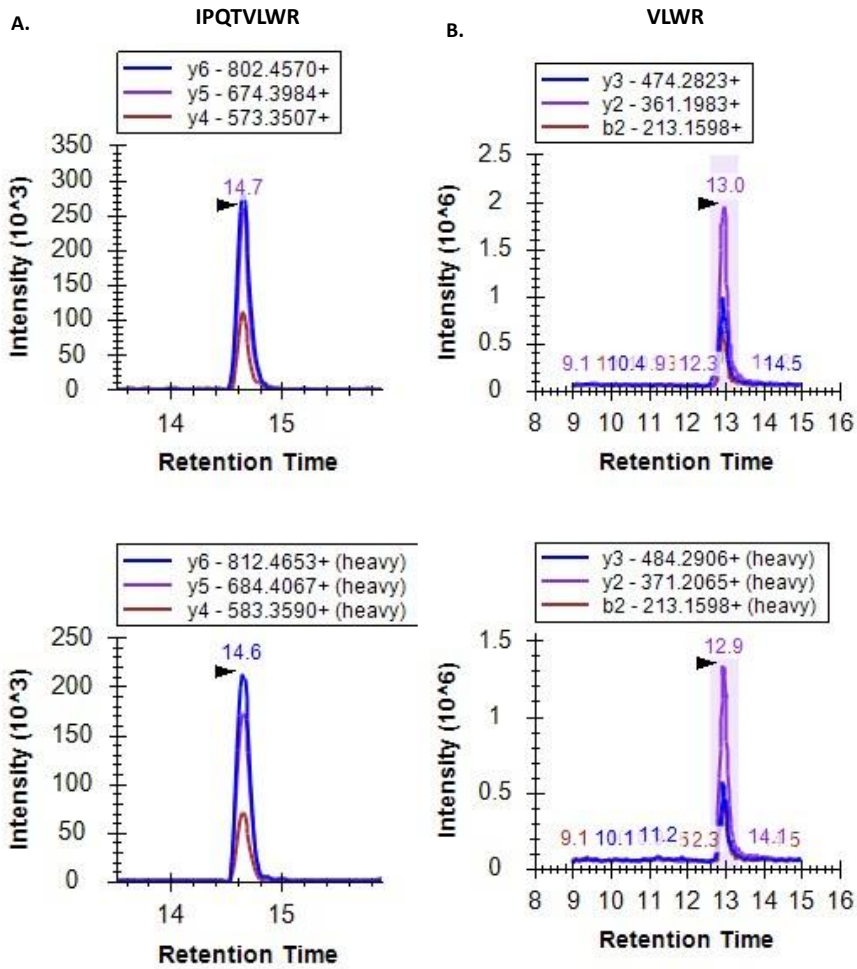
<b>Supplementary Table 3:</b> UGTs abundance (pmol/mg microsomal protein) in HLM, HIM, HKM, and HLS9				
UGT isoform	HLM	HIM	HKM	HLS9
UGT1A1	85.01 ± 4.42	9.65 ± 1.37	<LLOQ	14.14 ± 1.88
UGT1A3	12.57 ± 2.37	0.93 ± 0.04	<LLOQ	2.76 ± 0.08
UGT1A4	88.26 ± 8.63	<LLOQ	<LLOQ	10.73 ± 0.64
UGT1A6	26.92 ± 1.87	<LLOQ	47.98 ± 7.00	3.01 ± 0.38
UGT1A7	<LLOQ	<LLOQ	<LLOQ	<LLOQ
UGT1A8	<LLOQ	<LLOQ	<LLOQ	<LLOQ
UGT1A9	31.14 ± 2.45	<LLOQ	69.69 ± 12.16	5.95 ± 0.43
UGT1A10	<LLOQ	6.26 ± 0.1	<LLOQ	<LLOQ
UGT2B4	71.09 ± 3.48	<LLOQ	<LLOQ	11.49 ± 0.40
UGT2B7	112.83 ± 6.08	16.54 ± 6.39	59.77 ± 2.10	20.92 ± 1.07
UGT2B10	76.46 ± 16.04	<LLOQ	<LLOQ	10.92 ± 0.20
UGT2B15	75.05 ± 5.13	<LLOQ	<LLOQ	9.76 ± 0.28
UGT2B17	16.80 ± 4.97	52.23 ± 6.02	<LLOQ	2.59 ± 0.17

LLOQ: lower limit of quantification

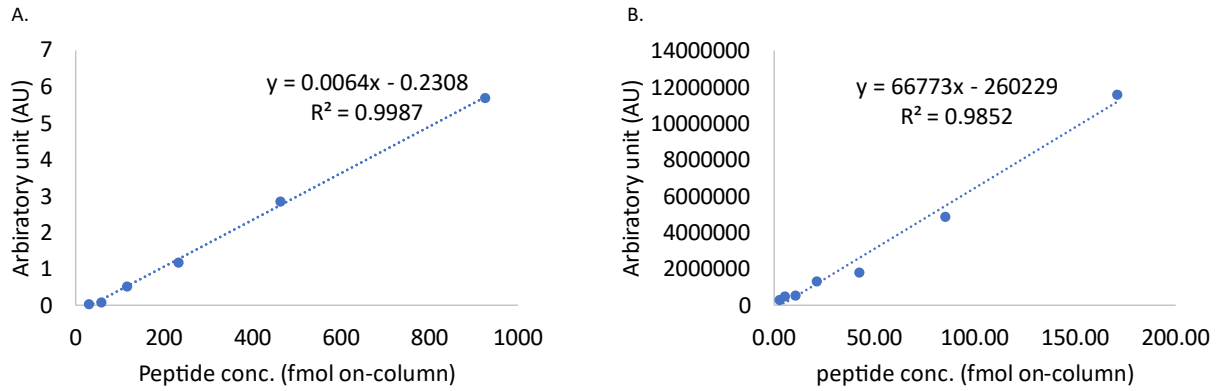




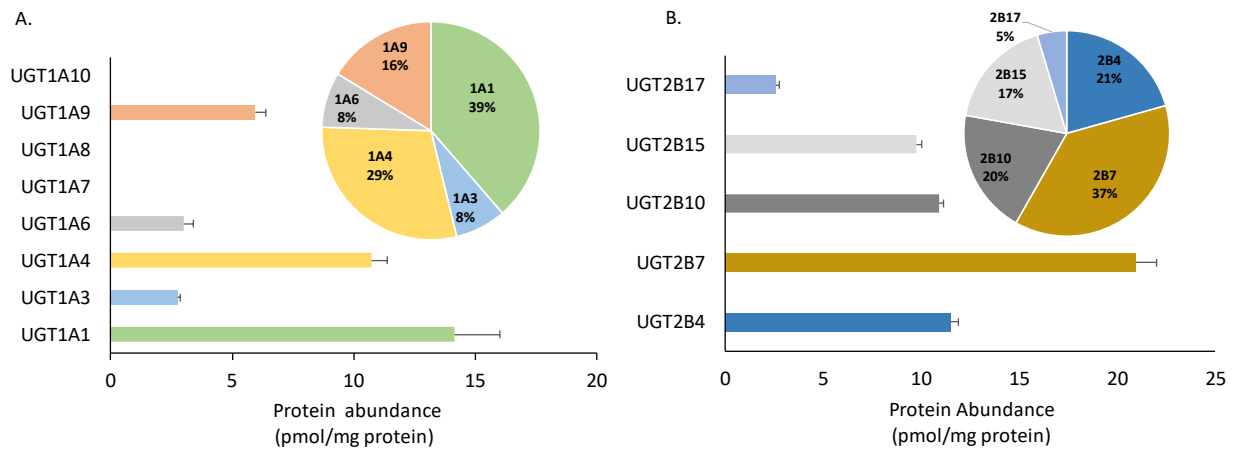
## Supplemental Figures



**Supplementary Fig. 1.** Representative chromatogram of two conserved peptides, IPQTVLWR (A) and VLWR (B) used in the quantification of UGT1A and UGT2B enzymes, respectively.



**Supplementary Fig. 2.** The calibration curves of the conserved peptides, IPQTVLWR (A) and VLWR, (B) were linear between 58 to 925 and 0.33 to 170.75 fmol on-column, respectively with  $R^2 > 0.98$ .



**Supplementary Fig. 3.** The abundance of UGT1As (A) and UGT2Bs (B) using the conserved peptide approach in the HLS9 fractions.

**Reference**

Bhatt DK, Mehrotra A, Gaedigk A, Chapa R, Basit A, Zhang H, Choudhari P, Boberg M, Pearce RE, Gaedigk R, Broeckel U, Leeder JS, and Prasad B (2019) Age- and Genotype-Dependent Variability in the Protein Abundance and Activity of Six Major Uridine Diphosphate-Glucuronosyltransferases in Human Liver. *Clin Pharmacol Ther* **105**:131-141.