Figure S1. A schematic of the *d*PC and its operation. The *d*PC consists of 41 gel plugs that differ by 0.05 pH units (pH 3.00-5.00 *d*PC is shown). The chip uses a discontinuous pH gradient and each gel feature has a distinct pH with the electrical field perpendicular to the operation of the *d*PC, i.e., the peptide transits in and out of the gel until it encounters a gel plug with a pH that equals its pI value.
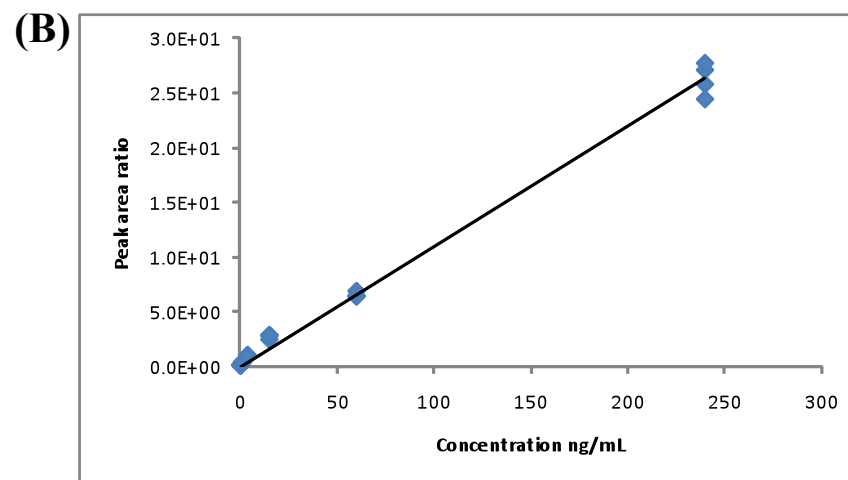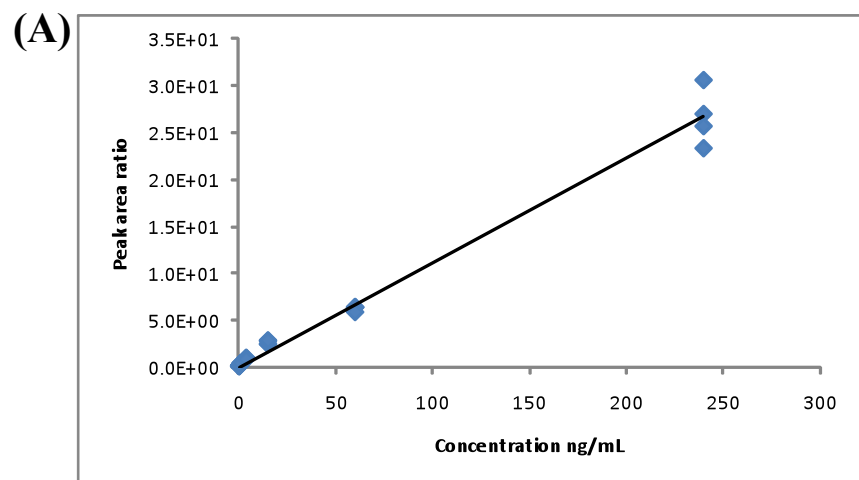
(A)

(B)

Figure S2. Calibration plots for PSA proteotypic peptides (range 0.12 – 240 ng/mL) (A) IVGGWECEK (r^2 = 0.9851), (B) LSEPAELTDAVK (r^2 = 0.9941). The peak area ratio of light to heavy peptide for different amounts of light peptide spiked into the depleted plasma digest was measured using the LC-Chip-SRM method and plotted versus the amount of light peptide.
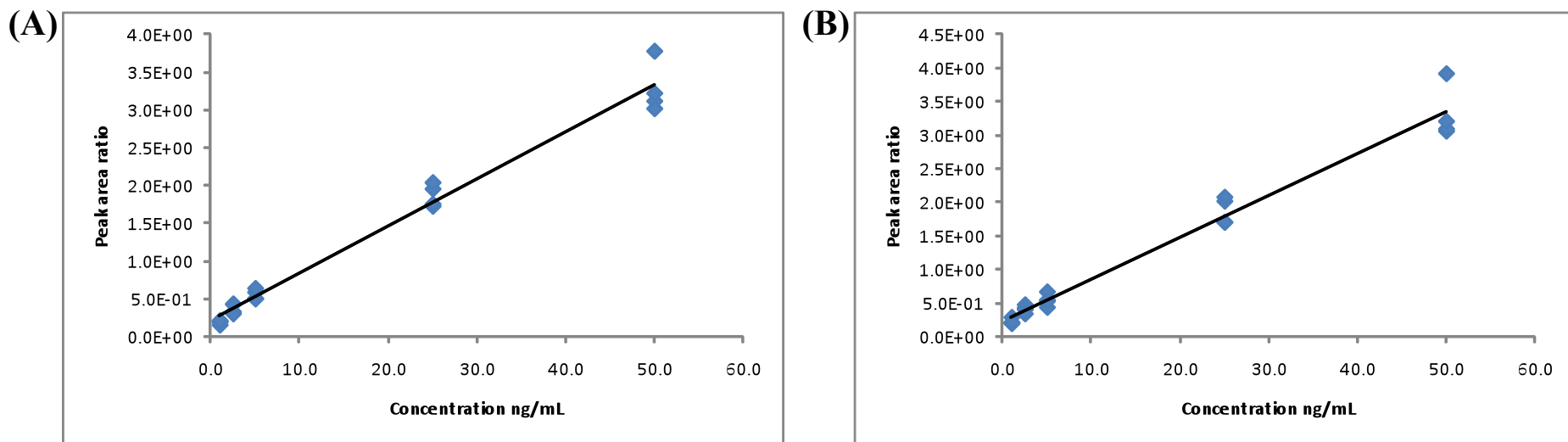
Figure S3. Calibration plots for quantitation of *d*PC enriched PSA peptides from clinical plasma samples (range 1.0 – 50 ng/mL) (A) IVGGWECEK ($r^2 = 0.9812$) and (B) LSEPAELTDAVK ($r^2 = 0.9752$). The peak area ratios of light to heavy peptide were measured using the LC-Chip-SRM method and plotted against the different concentrations of PSA standard protein added into female plasma prior to depletion of albumin and IgG.
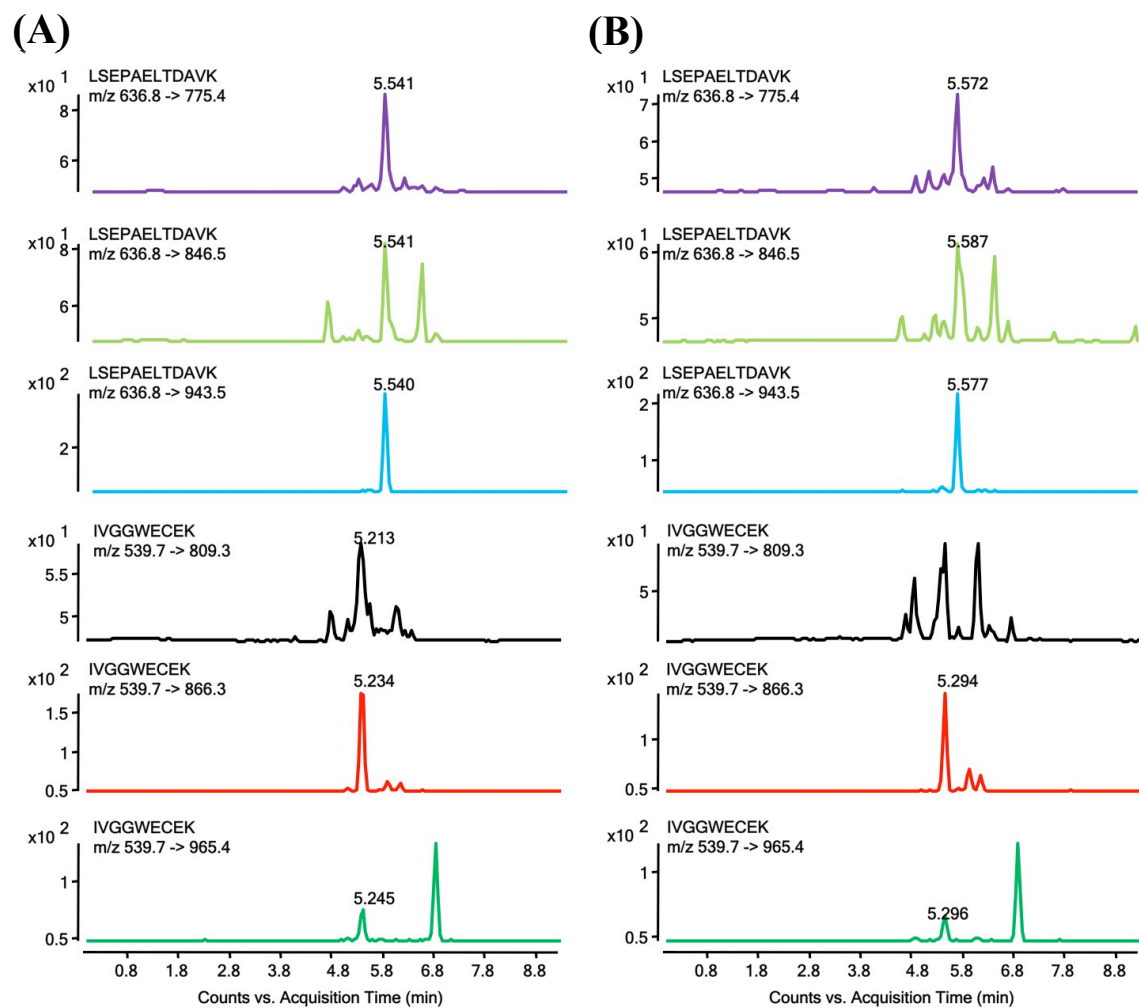
Figure S4. Comparison of the XICs acquired on all three SRM transitions for LSEPAELTDAVK (elution time 5.6 min) and IVGGWECEK (elution time 5.3 min) proteotypic peptides in (A) a patient with PSA level quantitated at 1.5 ng/mL and (B) the LOQ of 1.0 ng/mL for PSA protein added into plasma prior to depletion of albumin and IgG.

Table S1. Prostate specific antigen (PSA) concentrations measured in each sample using both methods[a].

Sample	ELISA (ng/mL)	Chip/Chip/SRM (ng/mL)
Patient 1	9.8	7.2
Patient 2	13	11
Patient 3	7.6	9.5
Patient 4	29	26
Patient 5	15	18
Control 1	4.1	1.5
Control 2	2.8	2.1
Control 3	2.2	2.8
Control 4	4.9	4.2
Control 5	1.5	2.1

[a]The PSA concentrations represent the average obtained for two measurements (technical replicates) of each clinical sample.