A New and Easy Approach for End-User Driven Quantification of Stable Isotope Labeled (SIL) Peptides in Targeted Proteomics

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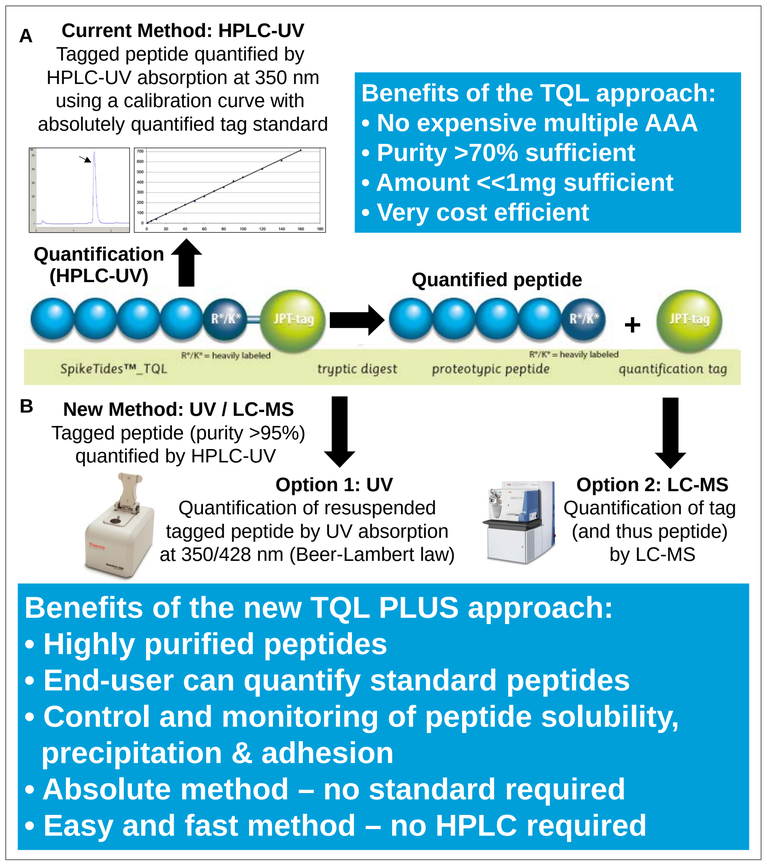
Introduction

Absolute protein quantification by targeted proteomics is dependent on the availability of stable isotope labeled (SIL) peptide standards. Recently, a new method for absolute peptide quantification has been developed (SpikeTide™_TQL concept). This method (outlined in Scheme 1A) relies on peptide quantification by HPLC-UV using a quantification tag (Qtag) with a specific UV absorption.[1-2]



Scheme 1: A) Concept of SpikeTides™_TQL quantification. B) Concept of the new SpikeTides™ TQL PLUS quantification.

Here we report on the extension of the approach in order to allow direct quantification of reconstituted UV-tagged peptides by the end-user (SpikeTides™ TQL PLUS concept, Scheme 1B). The method enables accurate quantification of peptide solutions within targeted proteomics workflows to control and monitor known problems related to peptide solubility, precipitation and adhesion to vials.

Scheme 1B shows the concept of the new approach. SpikeTide_TQL peptides are purified to a high level (purity >95%) and aliquoted to provide precise absolute amounts of peptide as dry powder. After reconstitution, the peptides can be re-quantified by:

Option 1: UV measurement at specific wavelength (quantity calculated based on Beer-Lambert law), and/or Option 2: LC-MS after tryptic cleavage of the Qtag (quantity determined relative to an absolutely quantified tag reference standard).

Methods and Results

To show the general applicability and accuracy of the method, different model peptides (Appendix) were prepared in high purity (>95%) and absolutely quantified by the standard methodology (HPLC-UV *vs.* an absolutely quantified reference standard). Aliquots of 10 nmol of dry powder were prepared.

Option 1: Quantification by UV

The peptide aliquots were resuspended in different solvent systems that are frequently used for peptide reconstitution (Figure 1, solvent A, B or C). Absolute quantification was performed by measuring the UV absorption at specific wavelengths in a standard UV Spectrophotometer (NanoDrop™ 2000). Figure 1 depicts the obtained quantification results compared to the respective quantities obtained by amino acid analysis (AAA).

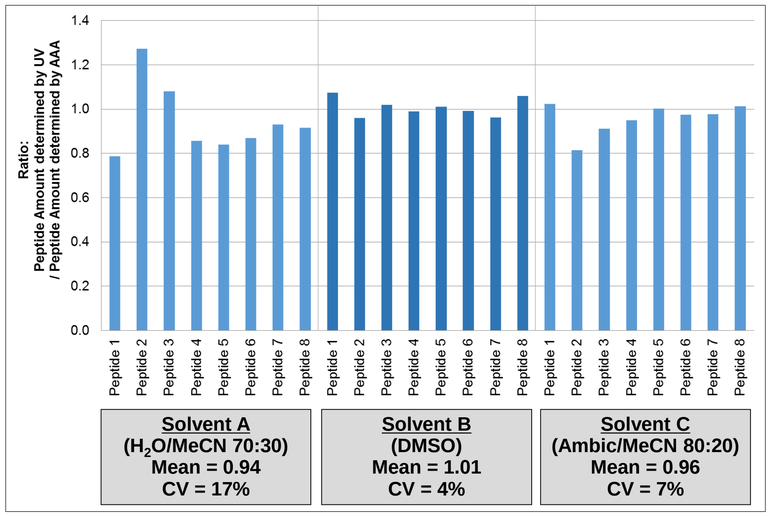


Figure 1: Quantification by UV compared to AAA.

Quantification by specific UV absorption yielded high concordance with quantities obtained by AAA. Depending on the solvent system used, mean values differed by only 0-6%, while the coefficient of variation (CV) ranged from 17% (H_2O/MeCN 70:30) to 4% (DMSO). These low values substantiate UV absorption as an efficient means for quantification of UV-tagged SIL peptide standards.

Option 2: Quantification by LC-MS

Several model peptides were absolutely quantified by the following procedure: a) Resuspension in solvent A/B/C, b) Tryptic digestion, c) Quantification of the obtained Qtag by LC-MS *vs.* an absolutely quantified heavy-labeled Qtag reference standard. The results (Figure 2) show a high consistency between the quantification results by the new method compared to AAA.

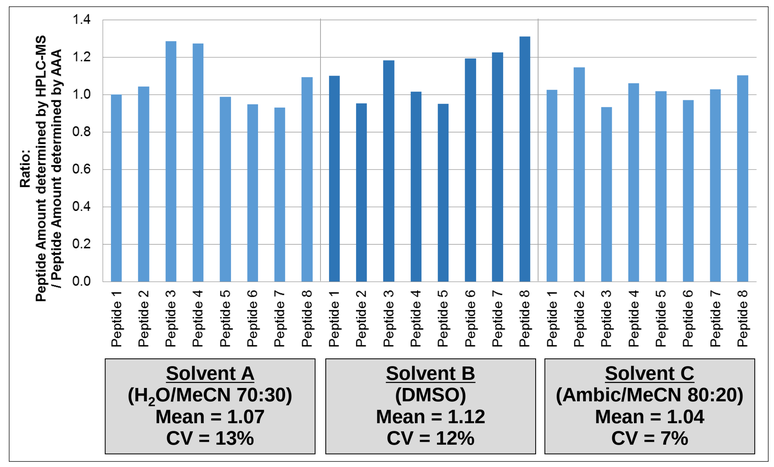


Figure 2: Quantif. of the released Qtag by LC-MS comp. to AAA.

Conclusion

A new and easy approach for end-user driven quantification of SIL peptide solutions in targeted proteomics was developed.

References

(1) Schnatbaum, K. et al. Non-peer-reviewed application-note in *Nat. Methods* **2011**, 8.
(2) Recent applications: e.g. (a) Simicevic, J. et al. *Nat. Methods* **2013**, *10*, 570-576. (b) Saito, M. A. et al. *Science* **2014**, *345*, 1173-1177. (c) Ebrahim, S. et al. *Nat. Commun.* **2016**, *1(7)*, 10833. (d) Korbakis, D. et al. *BMC Medicine* **2017**, *15:60*, 1-12. (e) Ermund, A. et al. *Biochem. Biophys. Res. Commun.* **2017**, *492(3)*, 331-337 (f) Yim, Y. Y. et al. *Biochemistry* **2017**, *56(40)*, 5405-5416. (g) Filippou, P. et al. *Clin. Biochem.* **2018**, *58*, 108-115. (h) Naggie, S. et al. *Clin. Proteomics* **2019**, asap (doi10.1002/prca.201800006)

Appendix

Table 1: Peptides used in the study.

Name	Sequence	Name	Sequence
Peptide 1	RPKYAGLTFPK*-Qtag	Peptide 5	EVKADDLEPIVELGR*-Qtag
Peptide 2	LNKIFDQLLLDR*-Qtag	Peptide 6	STKSSPESPVSGLQR*-Qtag
Peptide 3	TLKNFEVESLFQK*-Qtag	Peptide 7	TQRGPVYIGELPQDFLR*-Qtag
Peptide 4	MHRDVKPSNILVNSR*-Qtag	Peptide 8	AFGAAFPK*-Qtag

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