

# A Novel 6x5 Peptide Mixture for LC-MS/MS Performance Monitoring

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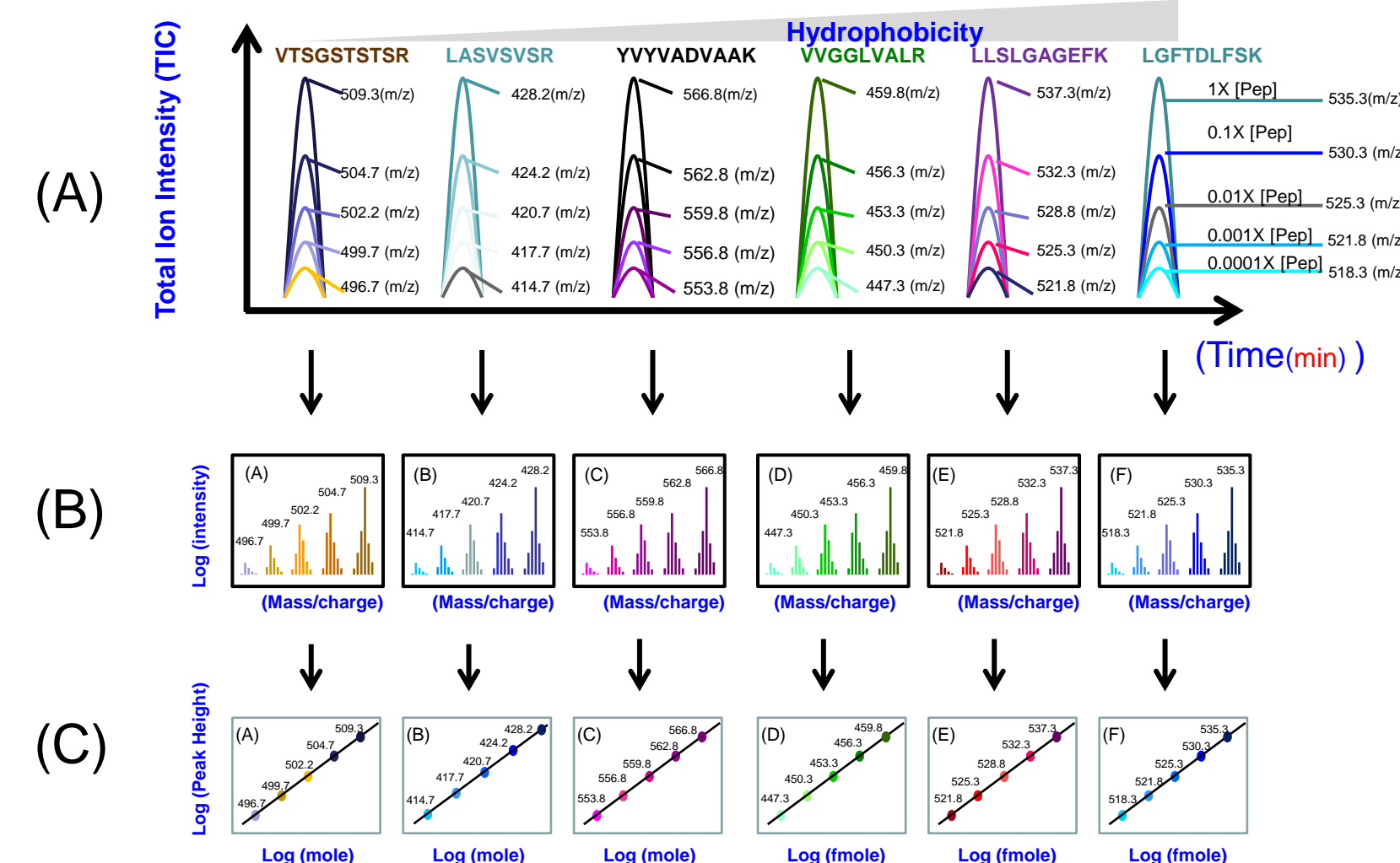
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## 1. Introduction

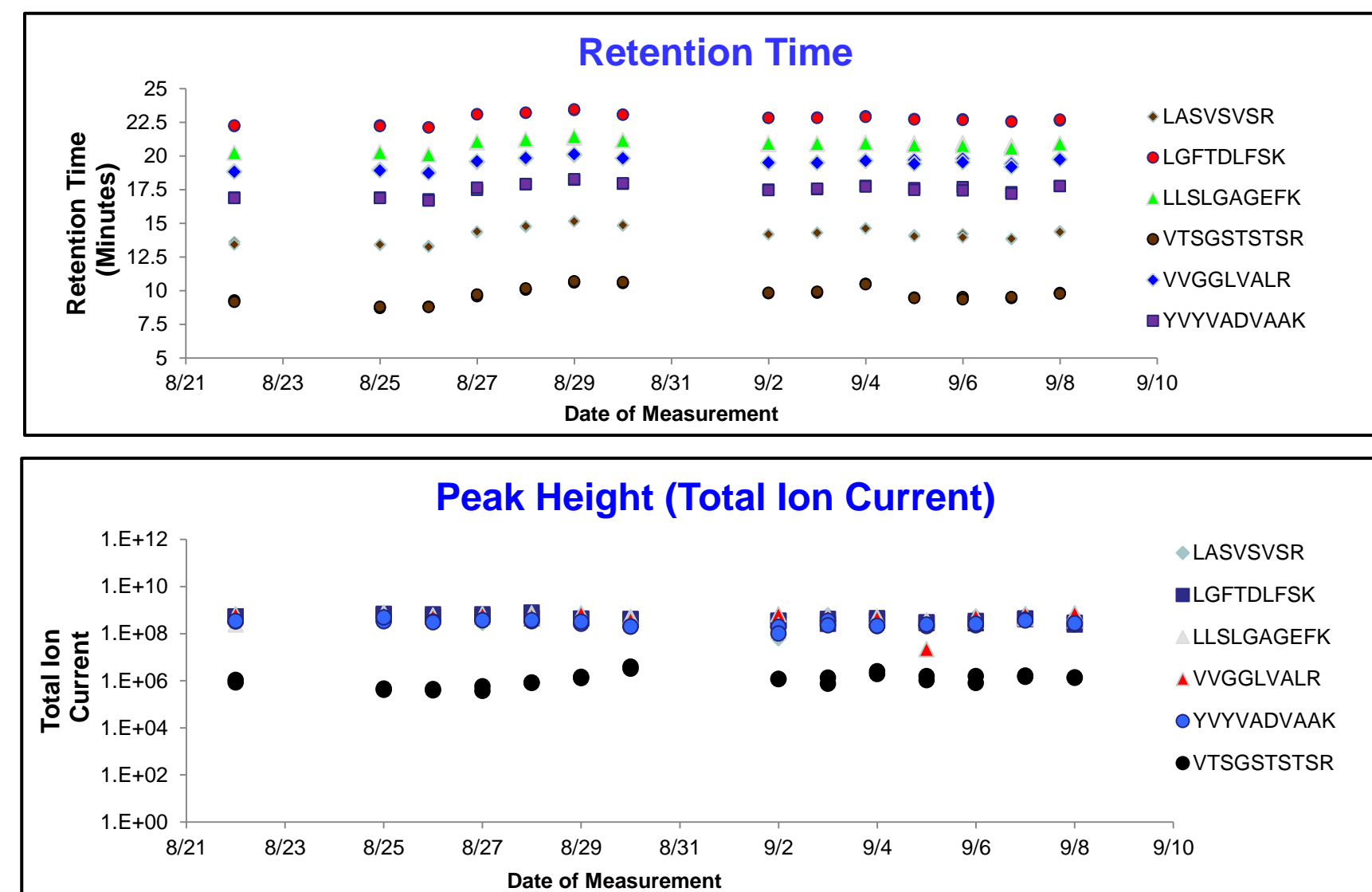
Performance monitoring of LC-MS/MS instrumentation continues to be challenging and has yet to be standardized across all MS laboratories. Here we report the development of a novel reference peptide mixture. In combination with an accompanying software tool, the mixture reports on LC column performance and MS instrument parameters, including sensitivity and dynamic range. In addition, the software can also report on parameter history, compare instruments, and display XIC traces for the peptides. This is the first example of a peptide mixture designed to give a report of all critical LC and MS parameters in a single run.

## 3. Monitoring LC and MS Parameters



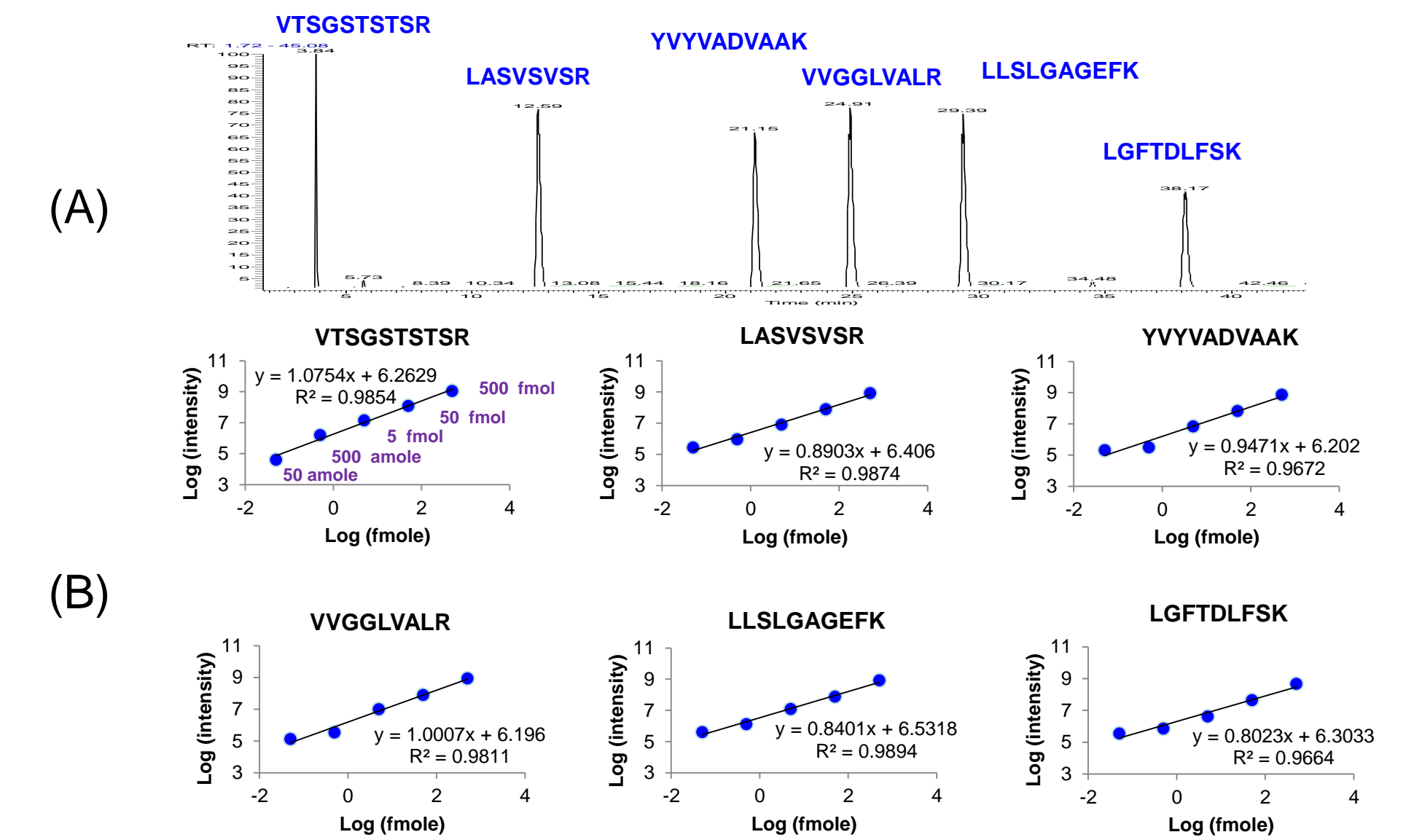
(A) In chromatography, 6 peptides elute, and each peptide is a mixture of 5 isotopologues. This can be seen in an XIC analysis, which will be available in the software. (B) MS spectra of the eluting peak. (C) A plot of the log of MS Intensity vs. the log of the molar amount on the column.

## 4. Instrument Parameter History



In addition to having individual LC and MS parameter reports, the software tool will also analyze parameters like Peak Height and Retention Time. Additional parameters like Mass Accuracy, Linear Fit, Lowest Detectable Mass, etc., can also be analyzed.

## 5. Chromatography and Mass Spec



(A) Representative chromatogram. (B) A plot of the log Peak Intensity vs. log (molar amount) provides a measure of the instrument sensitivity and dynamic range. Optimal loading conditions are first determined by titration to identify the region of optimal instrument linearity.

## 2. Software Analysis Tool (PReMiS)

Summary Report					
Summary					
Date and Time: Thursday, August 21, 2014 9:45:35 AM					
Sample Type: Neat					
Quantity of Peptide Loaded: 0.50 pmol					
Configuration: Promega MS Configuration					
Analyst Name: MikeR					
Raw Data File: MS817520_03.raw					
LC Data					
Peptide	Sequence	Retention Time (minutes)	Peak Width(sec)	Peak Height (MS counts; 10 <sup>-8</sup> )	
1	VTSGSTSTR	3.72	2.49	3.67E+09	
2	LASVSISR	13.69	10.03	1.66E+09	
3	YVYVADVAAK	22.53	10.01	1.97E+09	
4	VVGGVLVALR	26.72	10.32	1.78E+09	
5	LLSLGAGEFK	30.97	12.49	1.31E+09	
6	LGFTDLFSK	39.61	11.54	1.32E+09	
MS Data					
Peptide	Sequence	Linearity slope	Linearity R <sup>2</sup>	Smallest quantity of peptide detected (fmol)	Mass Accuracy (ppm)
1	VTSGSTSTR	1.025	0.996	0.050	-3.90
2	LASVSISR	0.947	0.994	0.050	-4.30
3	YVYVADVAAK	1.079	0.997	0.050	-2.85
4	VVGGVLVALR	0.888	0.995	0.050	-4.06
5	LLSLGAGEFK	0.849	0.982	0.050	-3.90
6	LGFTDLFSK	0.784	0.966	0.050	-4.11

Additional features: History Analysis, Multi-Instrument Analysis, and XIC analysis

## 6. Conclusions

- First system suitability (SS) tool to give all key LC and MS parameters in one experiment
- First software app tied to SS reagent making analysis quick and easy
- App provides tools to analyze trends and therefore troubleshoot as well as compare data across instruments
- The mixture is able to confirm up to 5 logs of dynamic range per run and is used to confirm the lowest detectable amounts per run (assuming initial proper titration, this can also be used to confirm LOQ and LOD)