



Standardizing, Comparing, and Translating Retention Times Across LC-MS Proteomic Platforms Using a Characterized Standard

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Overview

Objective: Demonstrate a quick, robust test of platform suitability, perform cross platform comparisons, and translate retention times across instrument setups.

Results We have developed a SIL peptide mix which (1) contains readily detectable, unique peptides that span the full elution profile of complex proteomic samples (2) provides a quick 'eye test' of whether instrument is performing to specification, (3) performs consistently over time in regards to peptide retention time and stability, and (4) can be used as a retention time translator across instrument platforms.

Introduction

Mass spectrometry based proteomic platforms require regular suitability checks and standardization in order to relate experimental data across and between analytical runs. Instrument performance assessment is especially critical when transferring assays or comparing data across platforms or laboratories. Additionally, in assay transfer and comparison, time and resources can be maximized when peptide target retention times can be accurately translated across platforms. We empirically determined Retention Factors (RF) for each peptide in the mix and subsequently of target peptides of interest. These retention factors, and a single injection of the 14 peptide mix on a different platform (or under differing conditions on the same platform) can then be used to translate retention times to targeted peptides on the alternate setups.

Composition of 14 peptide mix

Peptide #	Peptide Sequence	Amount (pmols)	Monoisotopic MW	Retention Factor
1	RGDSPASSP[K]	132	1008.5080	0.0
2	GLV[K]	309	423.2937	3.1
3	LGGNETQV[R]	81	982.5071	13.9
4	AEFAEVS[K]	101	887.4480	22.7
5	SGFSSVSVS[R]	67	1021.5068	30.3
6	ADEGISF[R]	76	903.4325	32.8
7	DISLSDY[K]	120	947.4691	39.0
8	LVNEVTEFA[K]	38	1156.6219	45.7
9	DQGGELLSL[R]	37	1096.5752	49.0
10	GLFIIDD[K]	100	927.5157	58.9
11	LGEYGFQNA[L]	82	1117.5517	63.1
12	YWGVASFQ[K]	61	1205.6324	75.2
13	TDELFIQIEGLKEELAYL[R]	350	2176.1291	86.7
14	AVQQPDGLAVLGLIFL[K]	46	1675.9752	100.0

Table 1. Amino acid in [brackets] denotes site of label incorporation as follows: [K], ¹³C₆¹⁵N₂ or [R], ¹³C₆¹⁵N₄ or [L], ¹³C₆

Elution Profile and LC-MS Platform Standardization

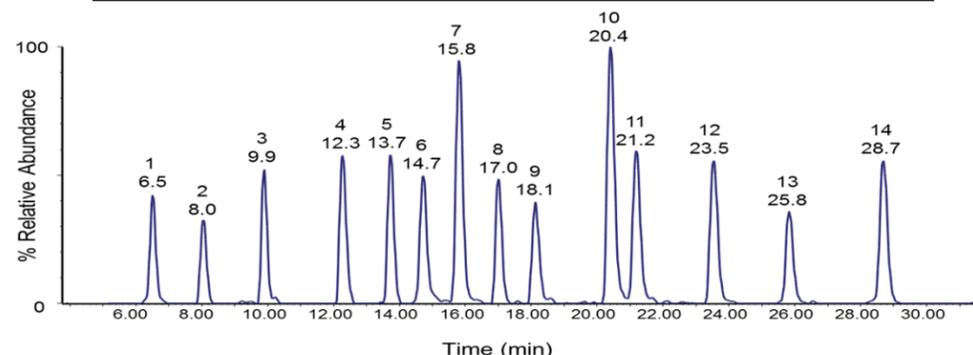


Figure 1. LC-MS TIC of 14 peptide mix (8 pmol load) using Waters Acquity UPLC and LCT mass spectrometer. Column: Supelco Ascentis Express Peptide ES C18 (1 mm I.D. x 15 cm long, 2.7 μm particles).

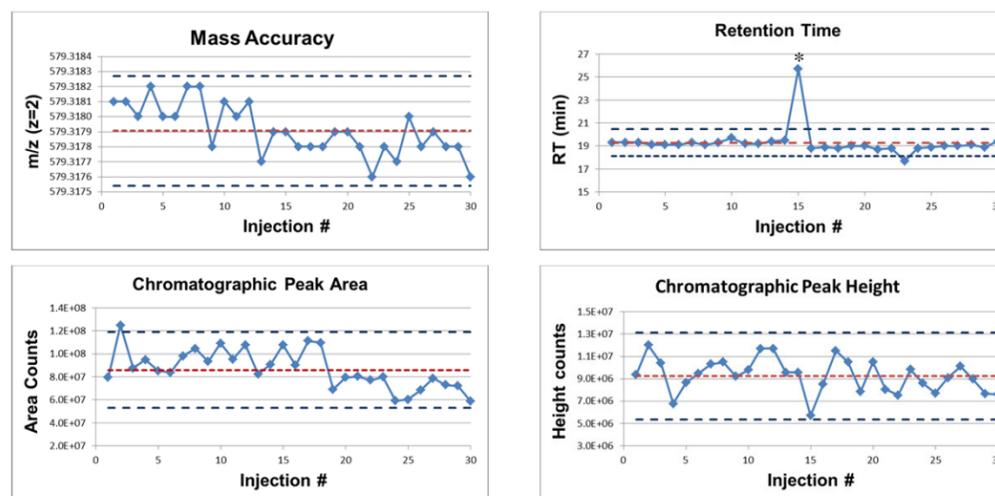


Figure 2. Statistical control charts (30 inj. over 3 mos.) of LC-MS parameters using Peptide LVNEVTEFA[K]. * For this data point it was determined that a partial column blockage occurred.

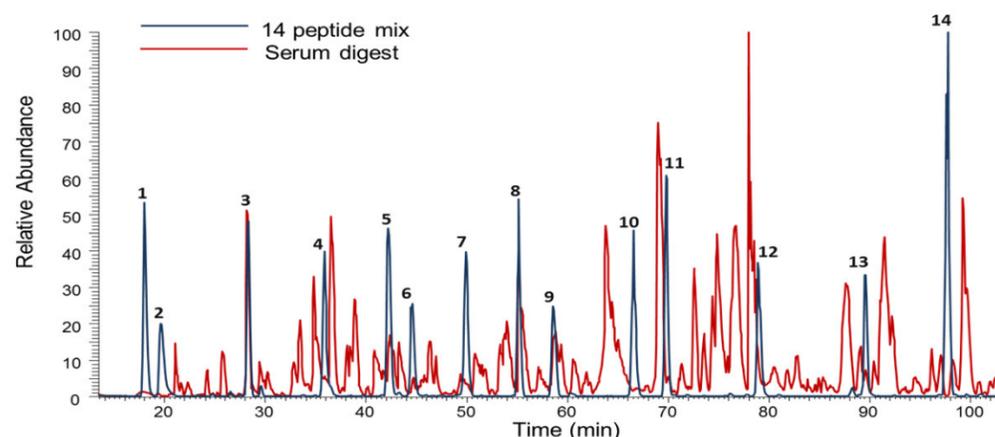


Figure 3. Overlay of TIC's from 14 peptide mix and a serum digest. Using similar platform as in Figure 1.

Retention Factor Determination and RT Translation

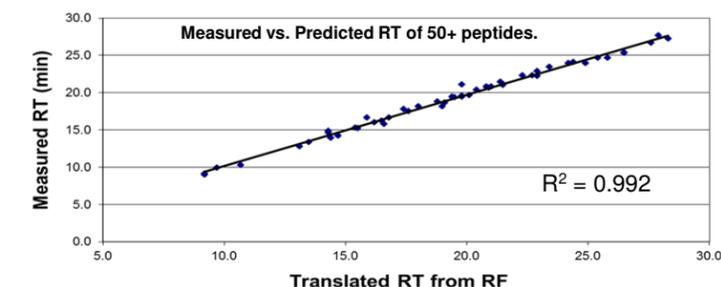
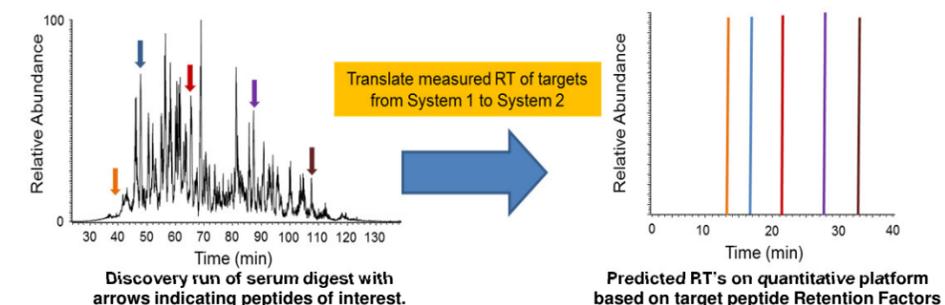
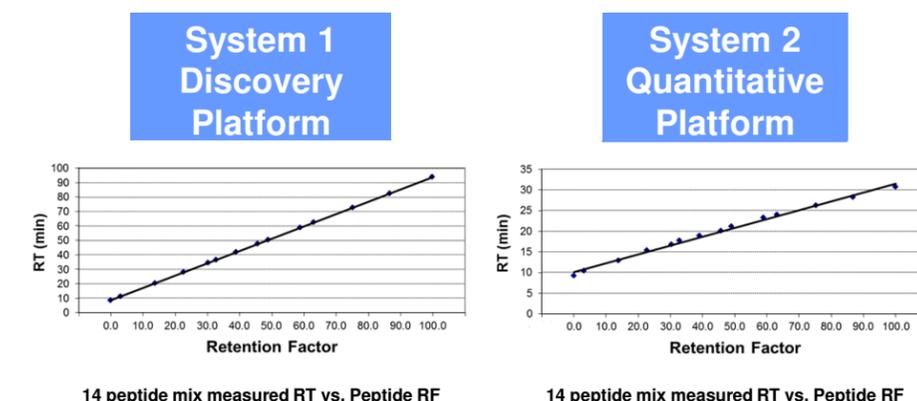


Figure 4. Overview of process flow for translating target peptide RT's from a discovery platform to a quantitative platform using 14 peptide mix as the translator.

Summary

- A 14 SIL peptide mix which spans the typical elution profile of a complex proteomic mix, was used to qualify LC-MS platform performance and accurately translate RT of target peptides of interest across instrument platforms.