An Improved Method for the Specific Isolation Of Phosphotyrosine-Containing Peptides

WenKui Lan McEldoon[1], Marc J. Horn[1],
Fan Xiang[2] and James Schilling[2]

[1]BioMolecular Technologies, Inc., Sunnyvale, California
[2]Sugen, Inc., South San Francisco, California

Introduction

We previously have described easy methodologies employing our *Pi*^{3}™ solid-phase reagents to selectively separate and isolate methionine-, tryptophan-, or phosphotyrosine-containing peptides from mixtures. We have applied these methodologies to digests of mixtures of proteins, peptides derived from in-gel digestion of proteins, as well as digestions of lysates. We report here the development of an improved, simplified and quicker method for isolation and enrichment of phosphotyrosine peptides from peptide mixtures, using our *Pi*^{3}™ Phosphotyrosine-QE solid-phase reagent.

Phosphotyrosine-Specific Isolation

The *Pi*^{3}™ Phosphotyrosine-QE Reagent (*Pi*^{3}-pTyr-QE) provides for the isolation and enrichment of phospho-tyrosine peptides from peptide mixtures, protein digests, and from mixtures containing phosphoserine- and phosphothreonine- peptides. These separated phospho-tyrosine-containing peptides subsequently may be regenerated and analyzed by mass spectrometry or liquid chromatography. Sites of tyrosine phosphorylation may be readily identified in known and unknown proteins. This new methodology has been shown to allow for the identification of phosphotyrosine peptides at the 2% occurrence level in mixtures.

Phosphopeptide General Isolation

Using the *Pi*^{3}-pTyr-QE Reagent

While providing for the isolation and enrichment of phosphotyrosine peptides from peptide mixtures, the *Pi*^{3}-pTyr-QE can also be used to isolate phosphopeptides in general. Using the same *Pi*^{3}™ solid-phase reagent, slight modifications in conditions will allow phosphophoserine, phosphothreonine and phosphotyrosine peptides to be isolated from mixtures. The separated phosphopeptides subsequently may be regenerated and analyzed by mass spectrometry or liquid chromatography. Compared with a commercially available gallium IMAC reagent, the *Pi*^{3}-pTyr-QE reagent provides substantially higher phospho-peptide yields, a more reproducible affinity for the phosphopeptides and lower non-specific binding.

Method for Use of *Pi*^{3}-pTyr-QE

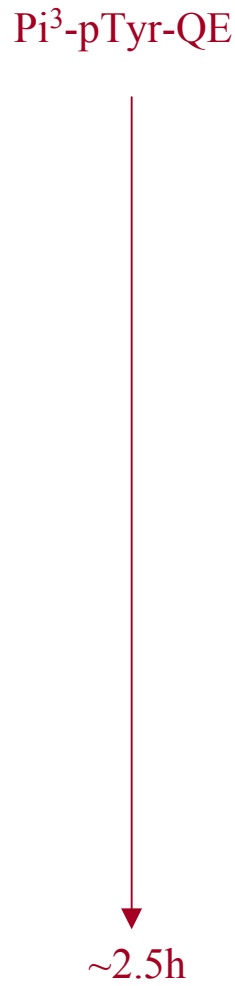
Step 1: Capture peptides on Pi^3 Reagent

Step 2: Wash to remove non-bound peptides

Step 3: Release bound peptides from Pi^3 Reagent

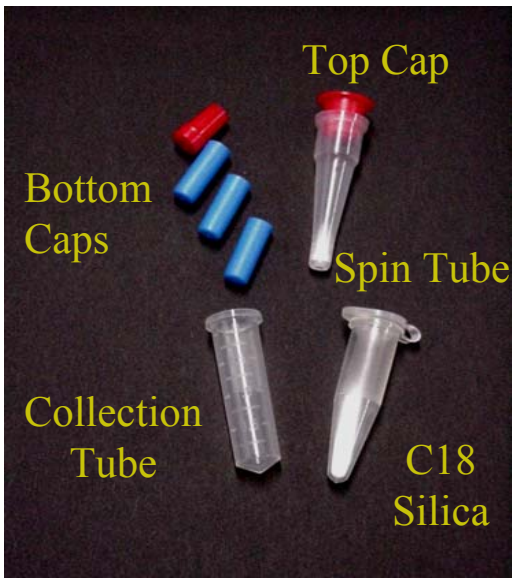
Step 4: Collect released peptides and wash Pi^3 Reagent

Step 5: Desalt and concentrate released peptide solution on Pi^3 clean-up column prior to analysis

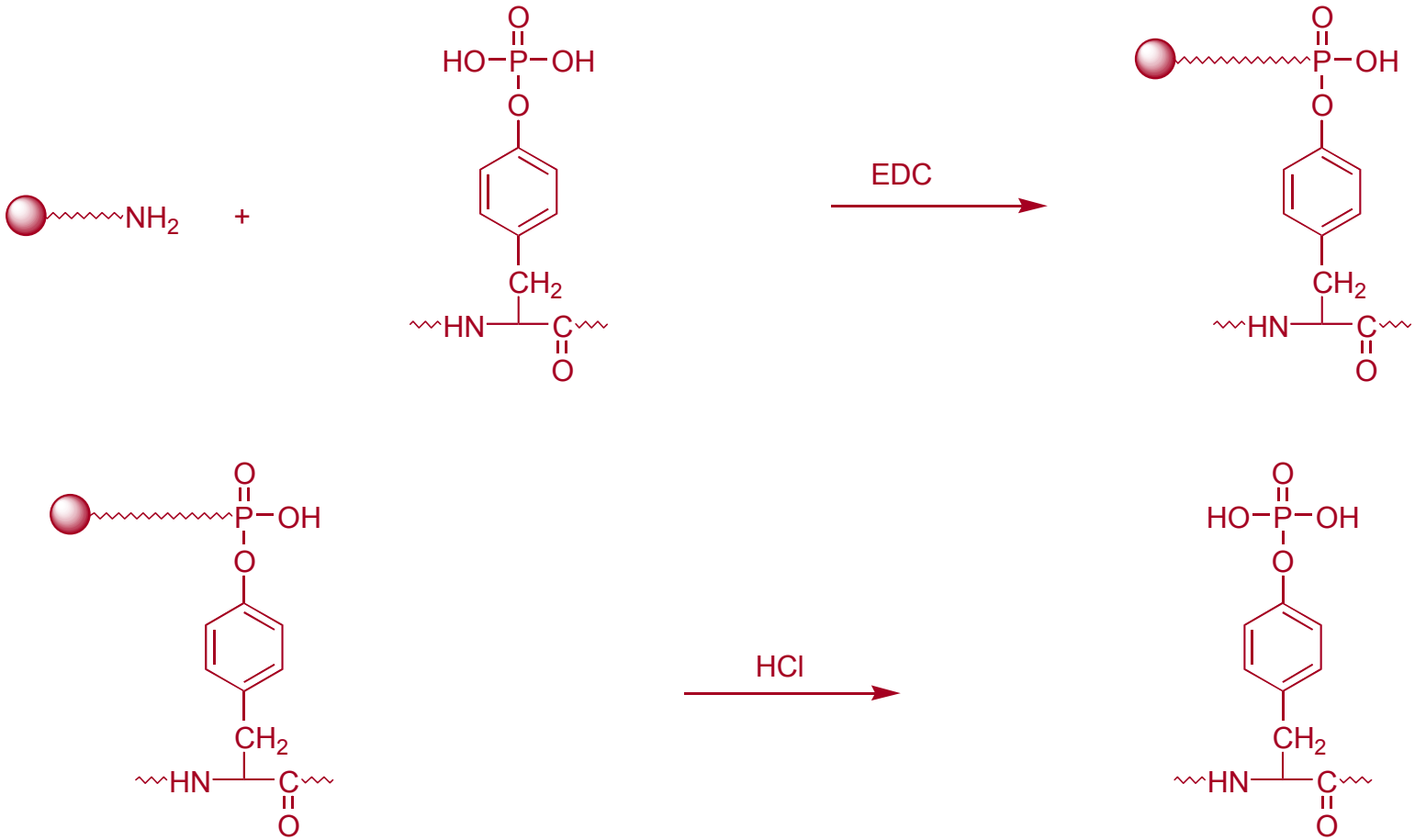


Pi^3 Methodology Equipment

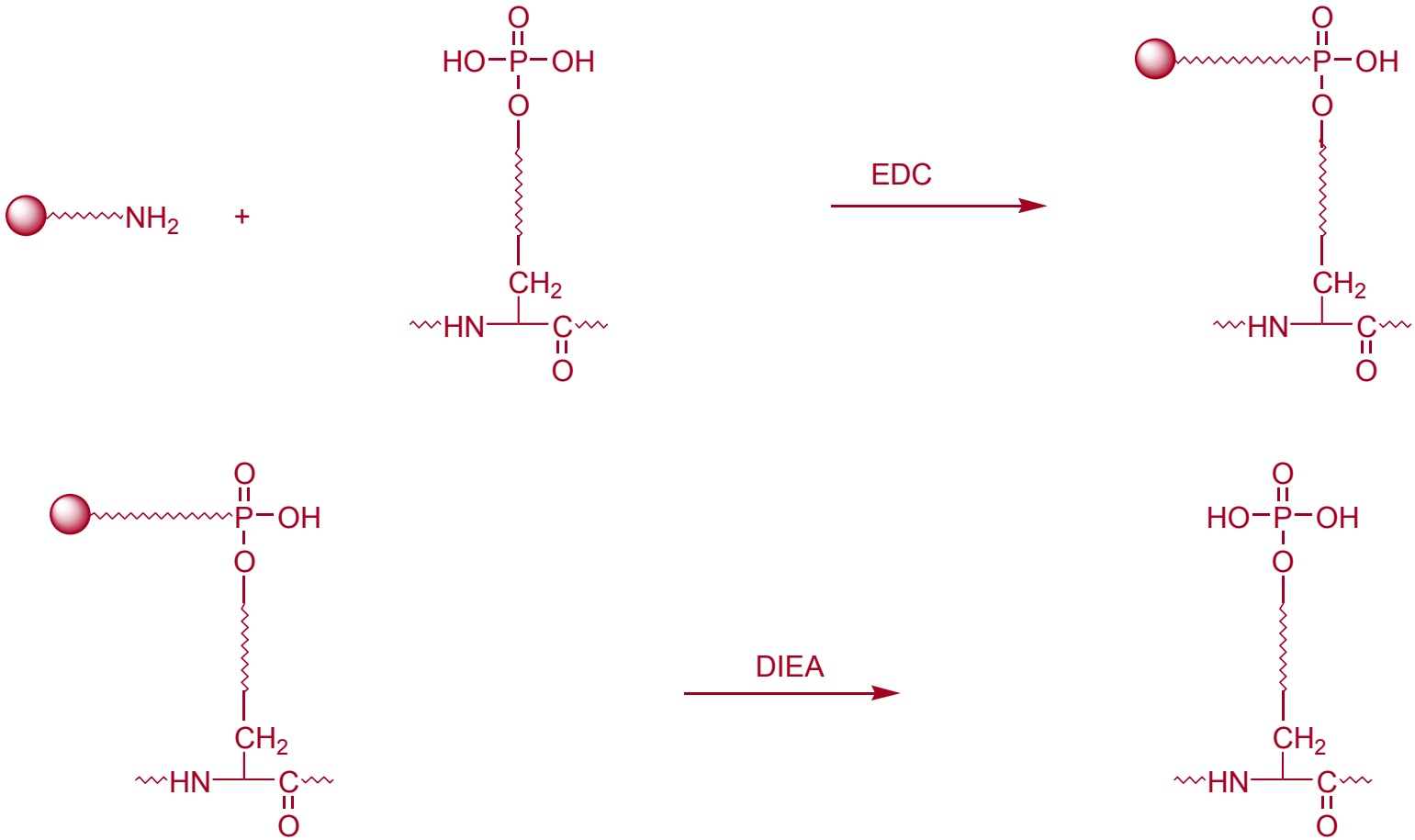
All steps of the procedure are carried out in a micro-spin tube. Reaction, mixing and washing steps are performed using a vortex mixer. A bench-top microfuge is used for separations and collection steps.



Phosphotyrosine-Specific Chemistry

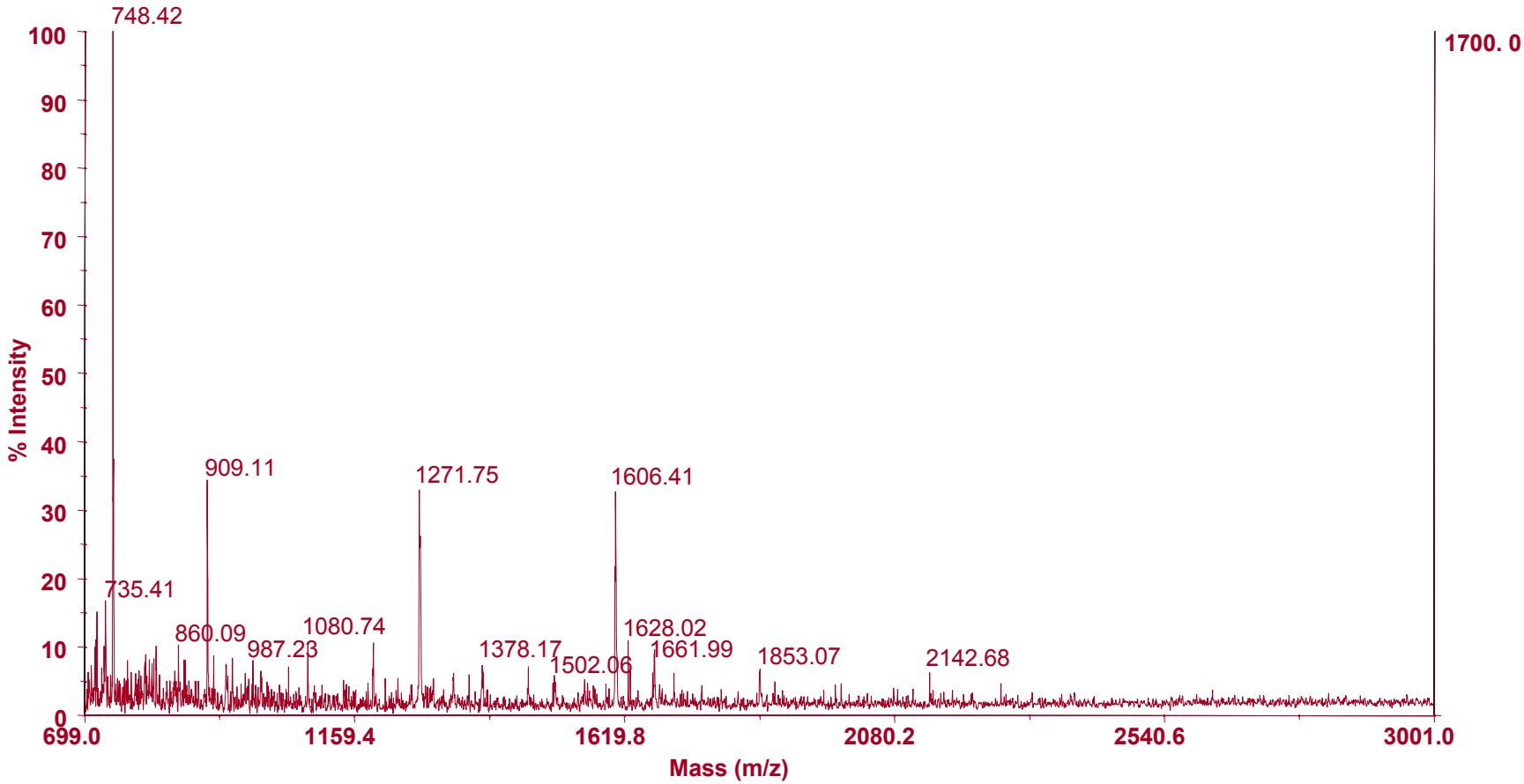


Phosphopeptide General Chemistry



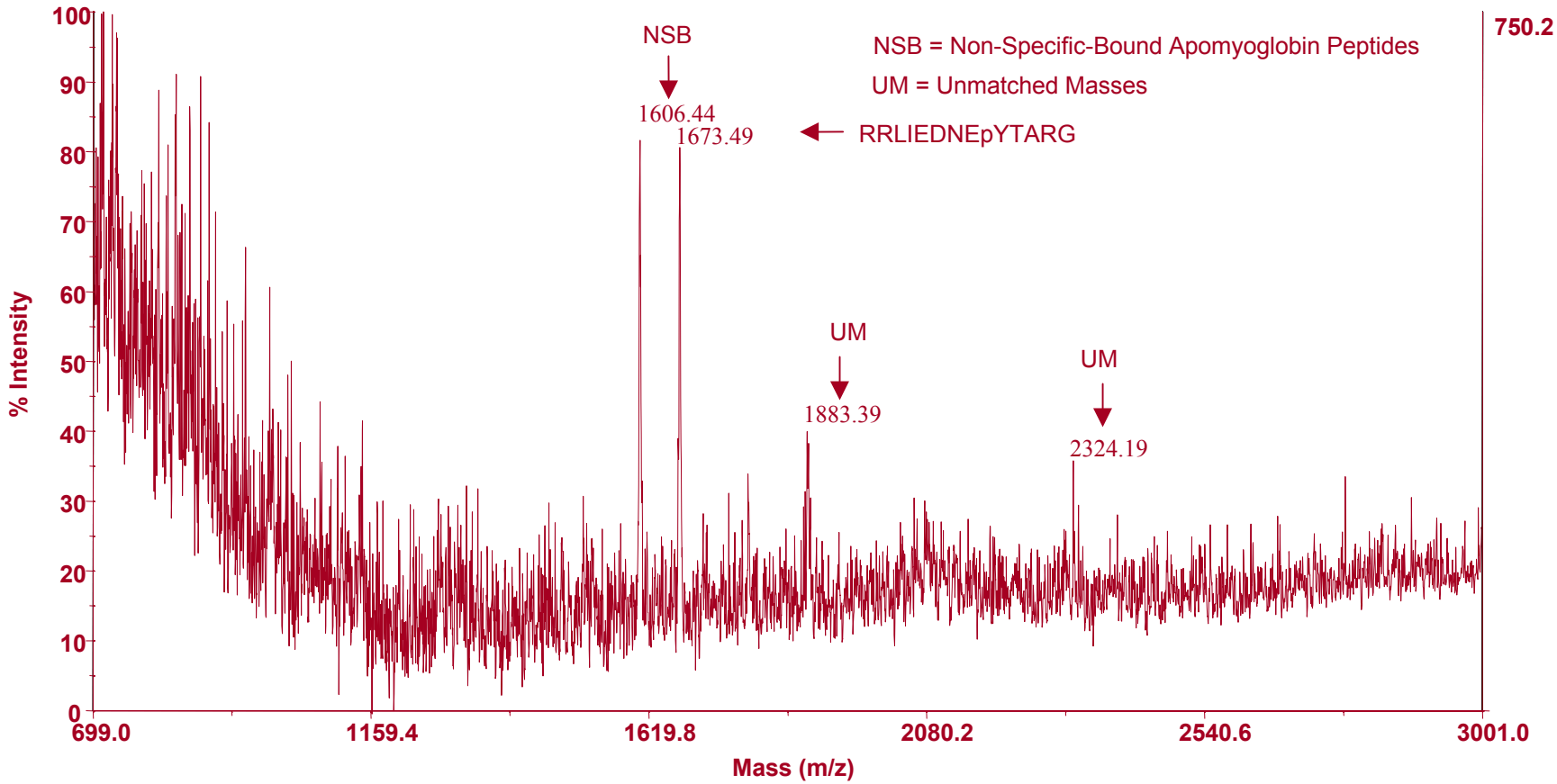
Apomyoglobin Digest w/Phosphopeptide Spike

5% Phosphopeptide (pS, pT, pY) Mix in 10 pmoles APO Tryptic Digest



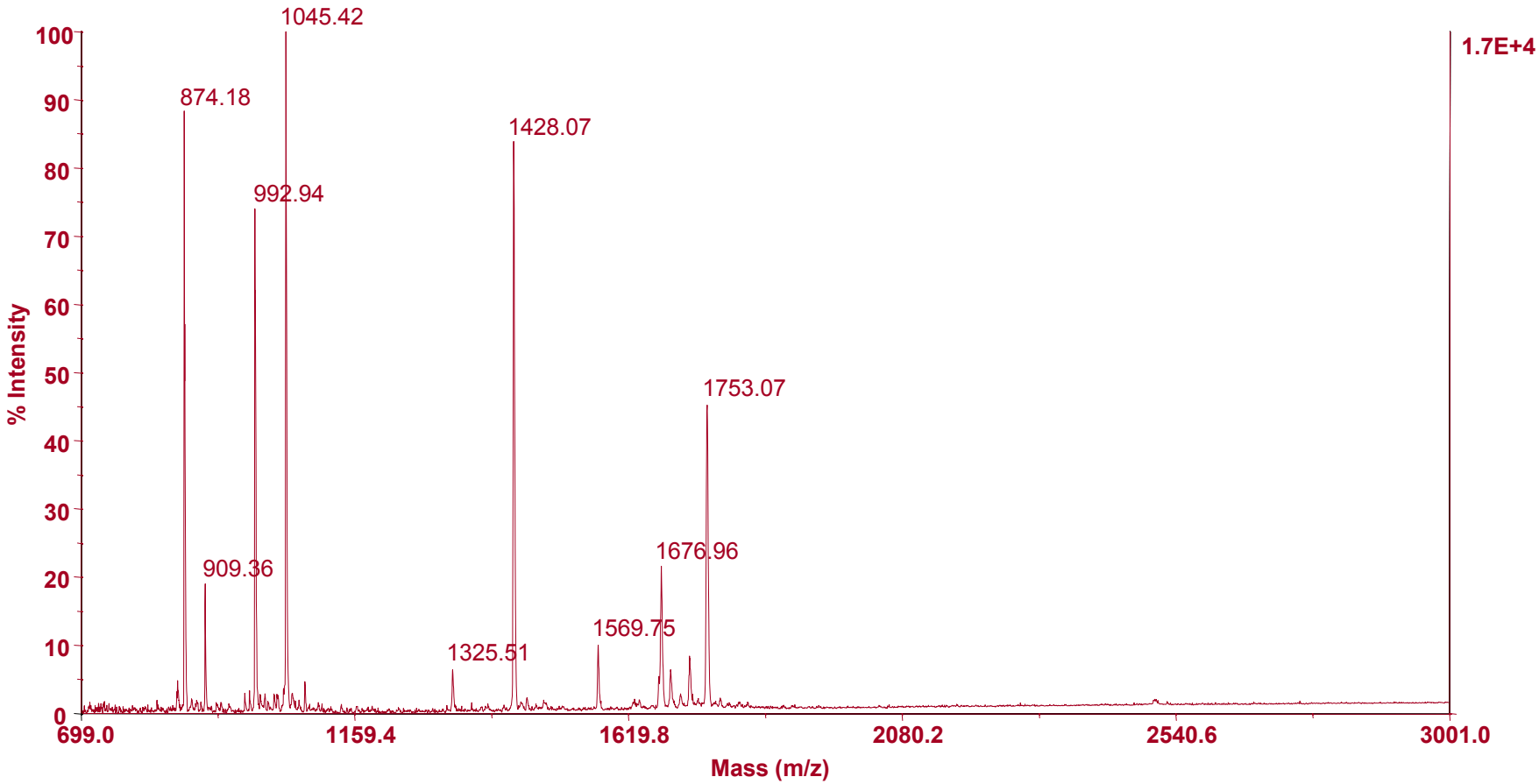
Pi^3 Phosphotyrosine Peptide-Specific Isolation

From 5% Phosphopeptide (pS, pT, pY) Mix in 10 pmoles APO Tryptic Digest



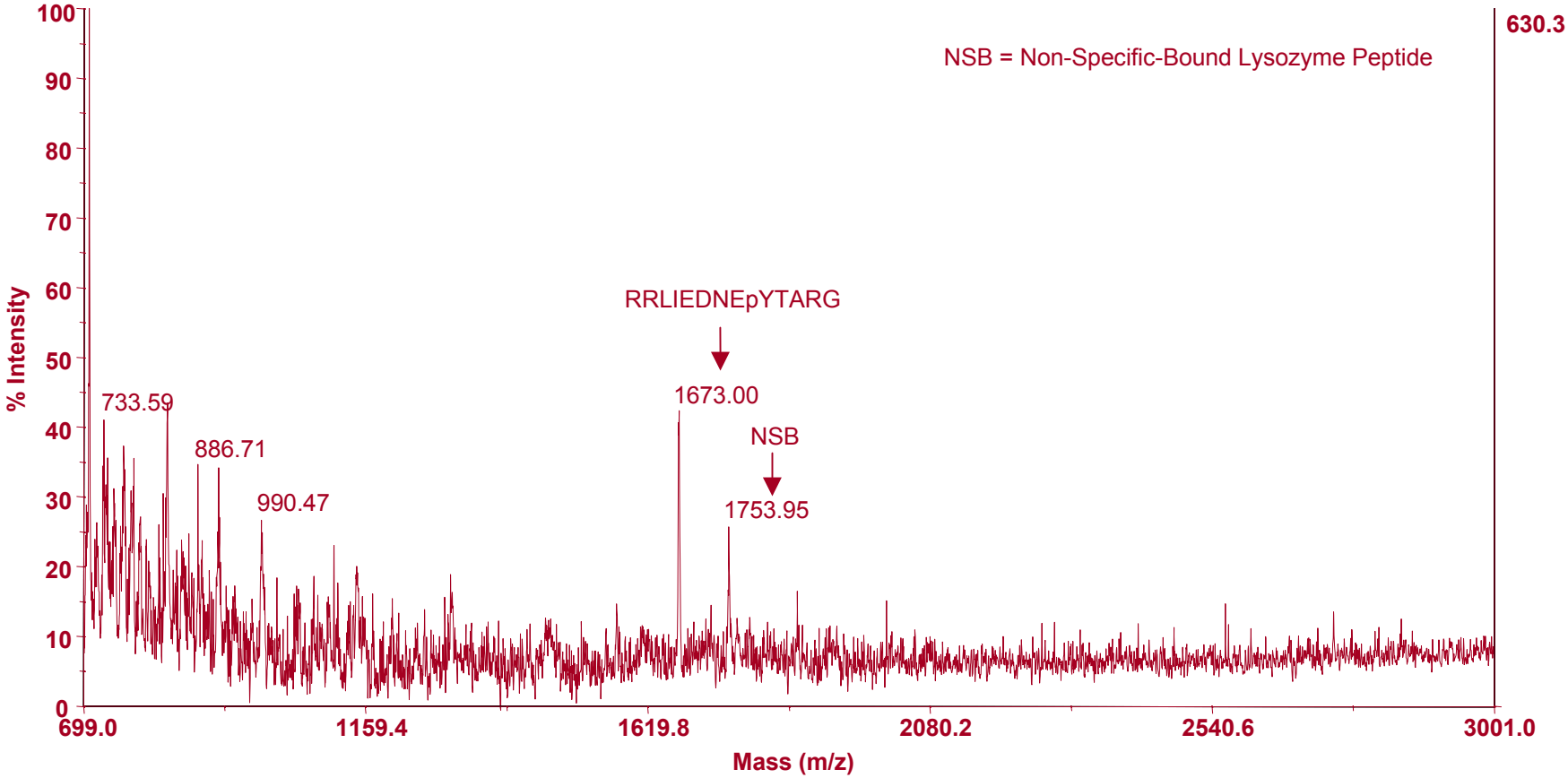
Lysozyme Digest w/Phosphopeptide Spike

2% Phosphopeptide (pS, pT, pY) Mix in 20 pmoles Lysozyme Tryptic Digest



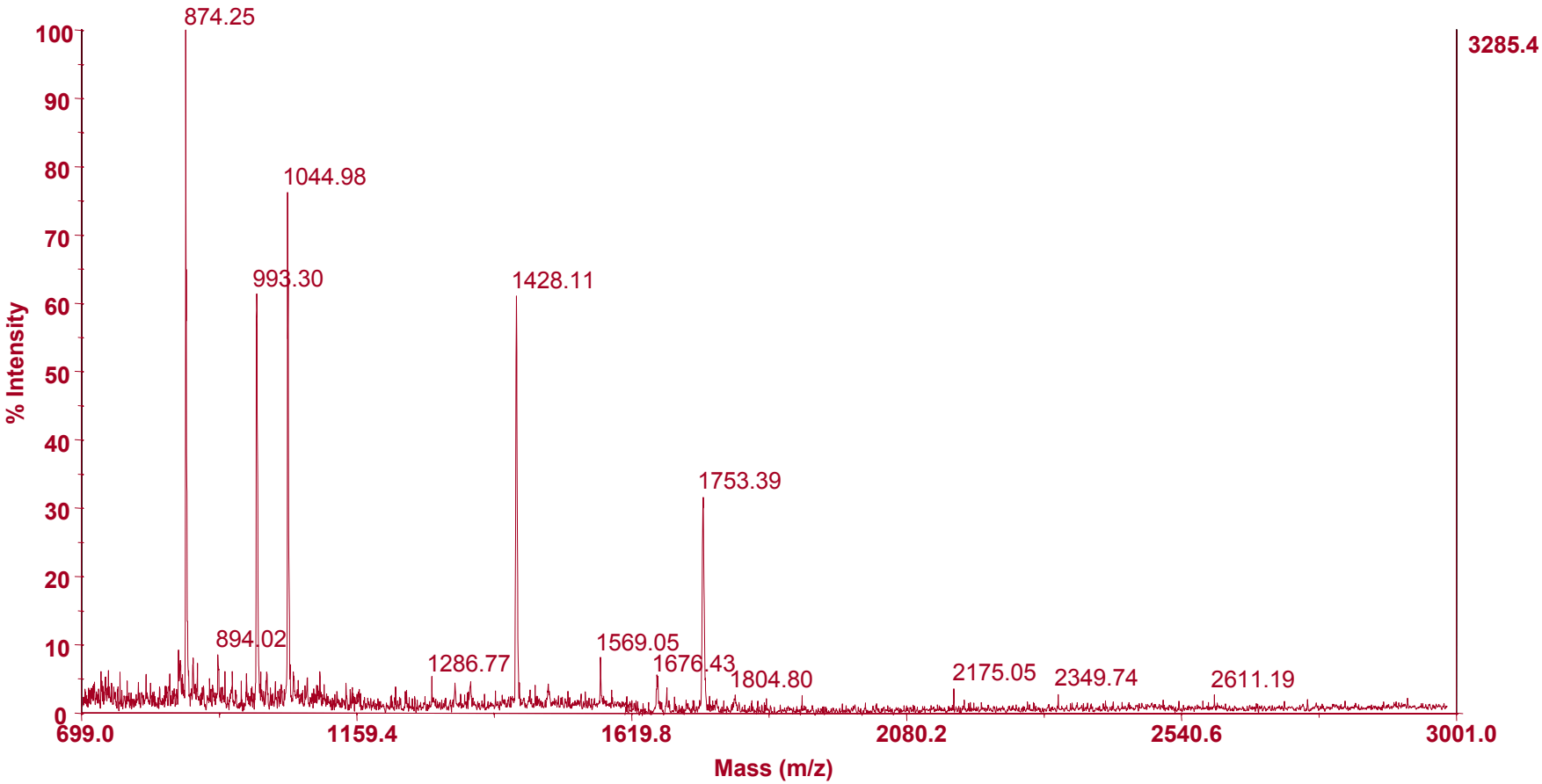
Pi^3 Phosphotyrosine Peptide-Specific Isolation

From 2% Phosphopeptide (pS, pT, pY) Mix in 20 pmoles Lysozyme Tryptic Digest



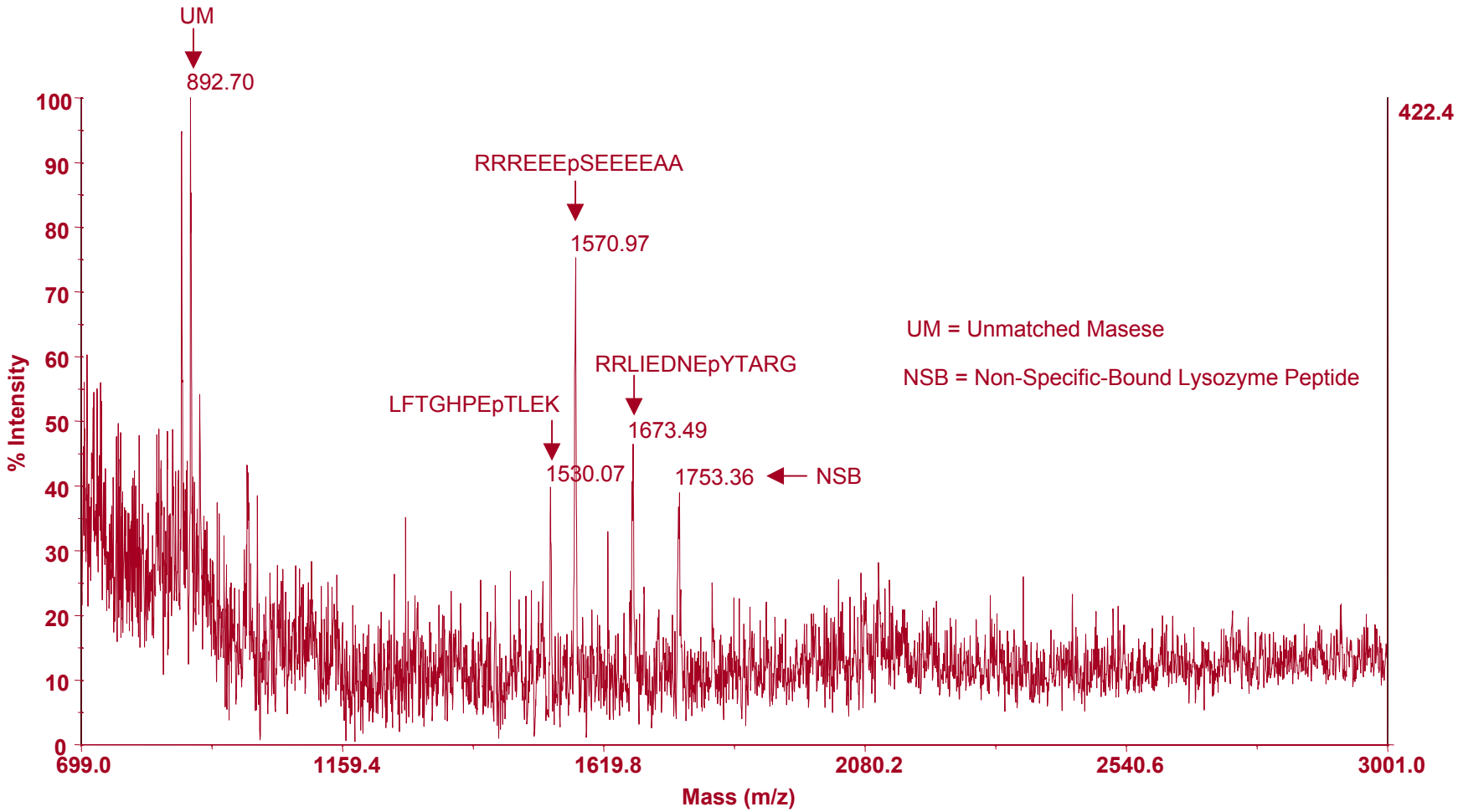
Lysozyme Digest w/Phosphopeptide Spike

2% Phosphopeptide (pS, pT, pY) Mix in 20 pmoles Lysozyme Tryptic Digest



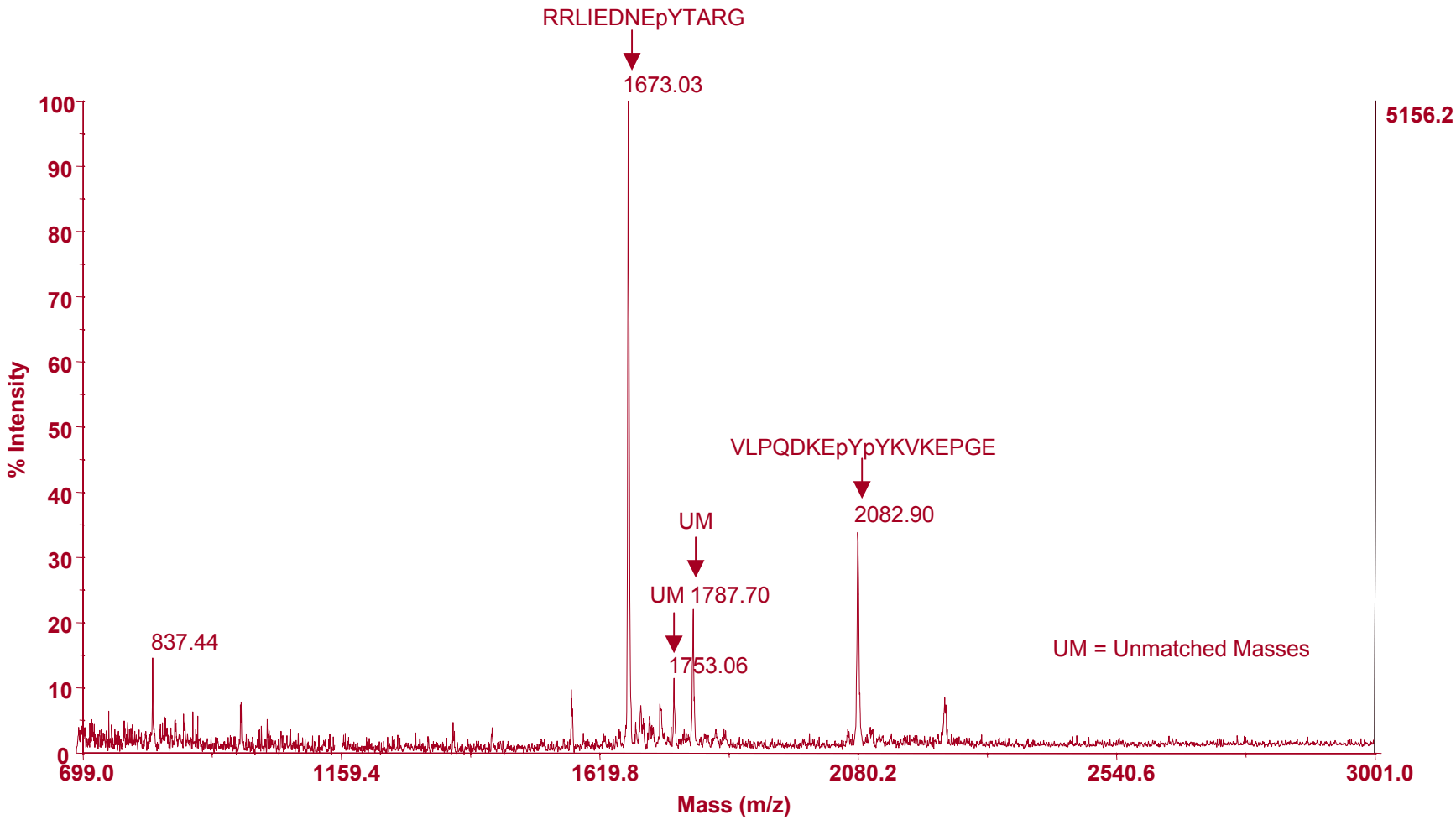
Pi^3 Phosphopeptide-Specific Isolation

From 2% Phosphopeptide (pS, pT, pY) Mix in 20 pmoles Lysozyme Tryptic Digest



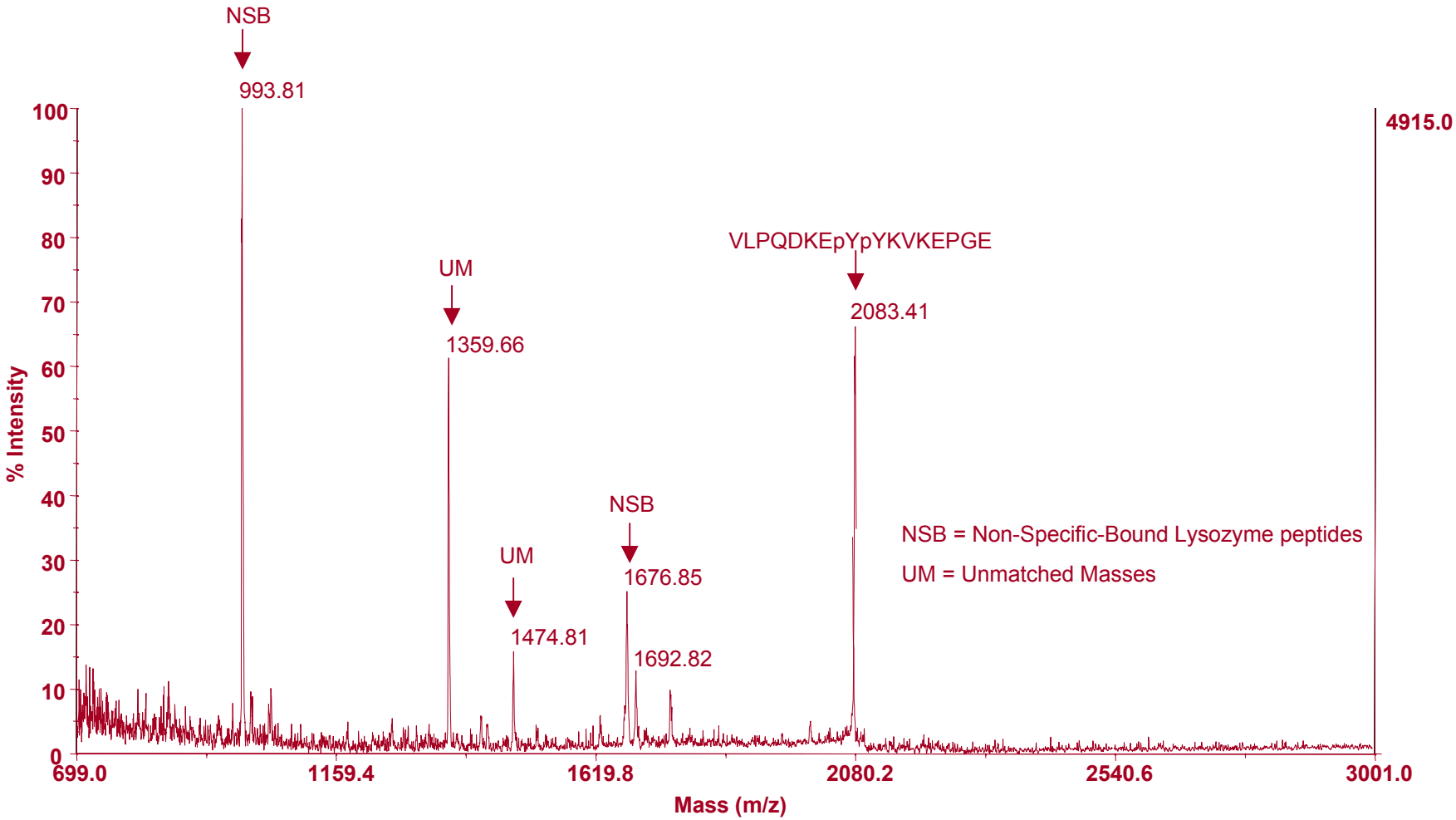
Pi^3 Phosphopeptide-Specific Isolation

From 50% Phosphotyrosine 2-Peptide Mix in 500 pmoles Lysozyme Tryptic Digest



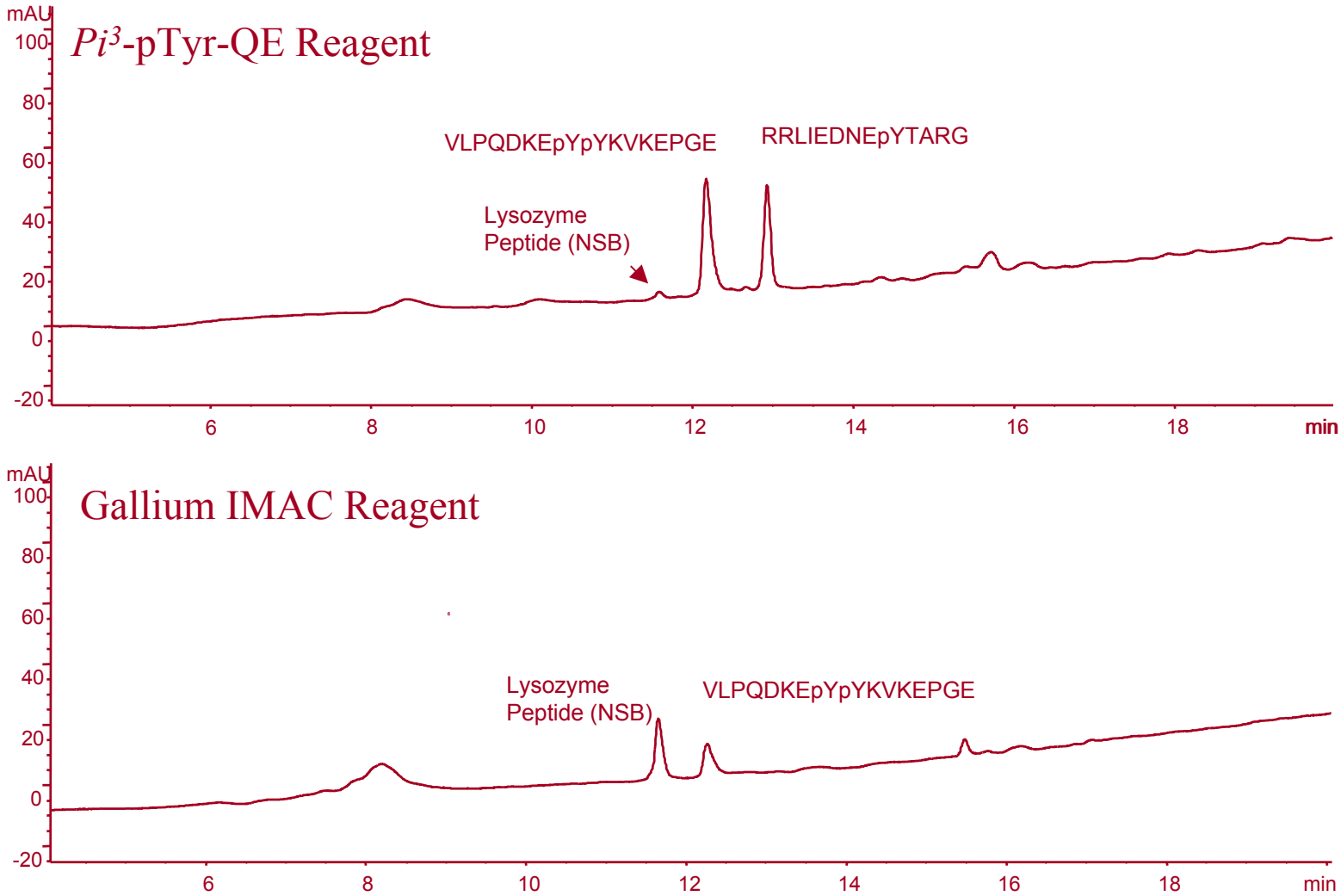
Gallium IMAC Phosphopeptide Isolation

From 50% Phosphotyrosine 2-Peptide Mix in 500 pmoles Lysozyme Tryptic Digest



Phosphotyrosine Peptide Isolation Efficiency

From 50% Phosphotyrosine 2-Peptide Mix in 500 pmoles Lysozyme Tryptic Digest



Phosphotyrosine Peptide Isolation Efficiency

From 50% Phosphotyrosine 2-Peptide Mix in 500 pmoles Lysozyme Tryptic Digest

Results

Pi^3 Reagent:

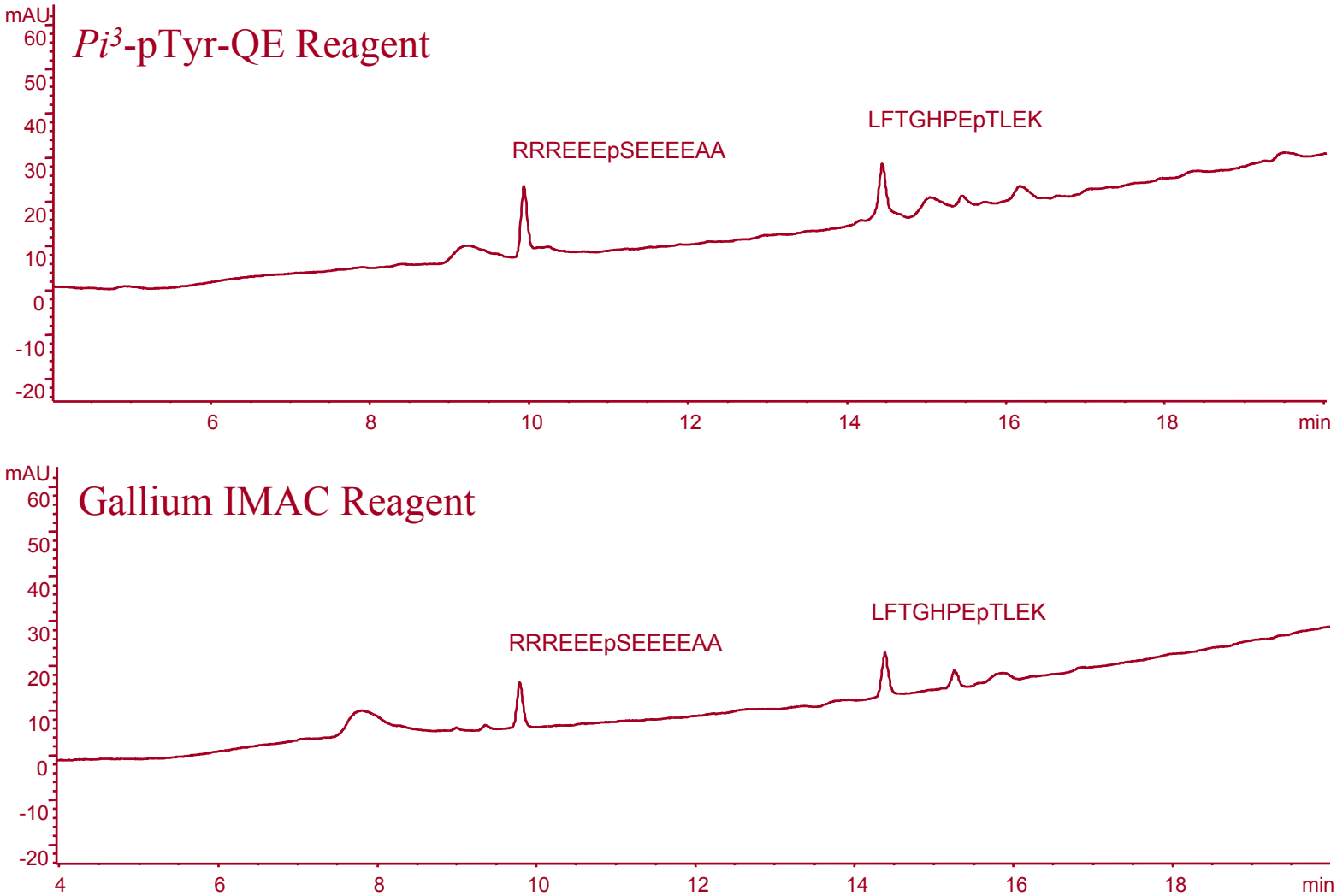
- VLPQDKEpYpYKVKEPGE recovery 83%
- RRLIEDNEpYTARG recovery 67%

IMAC Reagent:

- VLPQDKEpYpYKVKEPGE recovery 25%
- RRLIEDNEpYTARG recovery 0%
- Large amount of Non-specifically bound Lysozyme peptide

Phosphothreonine/serine Peptide Isolation Efficiency

From 50% Phosphothreonine/serine 2-Peptide Mix in 500 pmoles Lysozyme Tryptic Digest



Phosphothreonine/serine Peptide Isolation Efficiency

From 50% Phosphothreonine/serine 2-Peptide Mix in 500 pmoles Lysozyme Tryptic Digest

Results

Pi^3 Reagent:

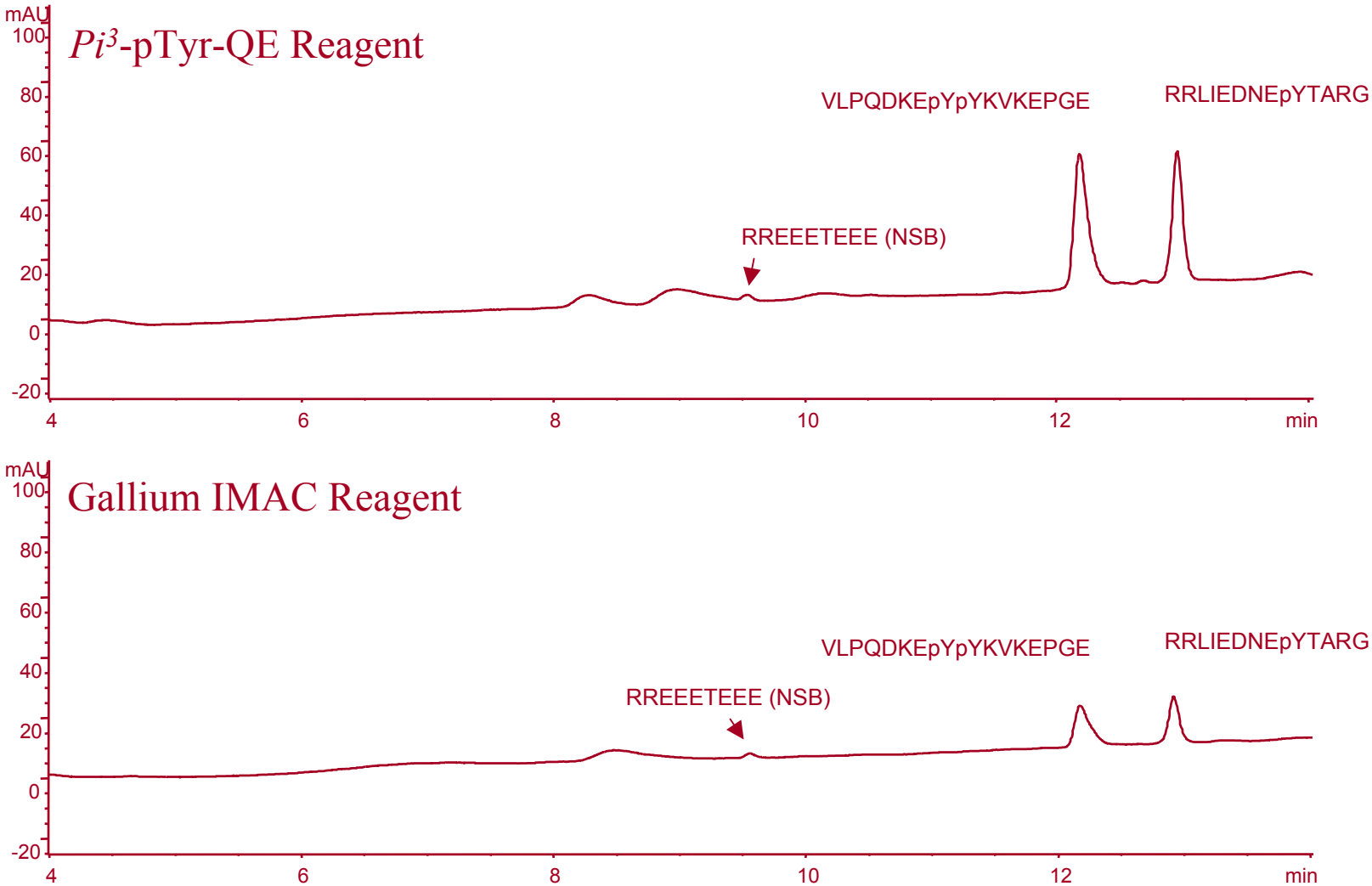
- RRREEEpSEEEEAA recovery 79%
- LFTGHPEpTLEK recovery 48%

IMAC Reagent:

- RRREEEpSEEEEAA recovery 53%
- LFTGHPEpTLEK recovery 37%

Phosphopeptide Isolation/Non-Specific Binding

From 250 pmole Phosphotyrosine /Acidic 3-Peptide Mix



Phosphopeptide Isolation/Non-Specific Binding

From 250 pmole Phosphotyrosine /Acidic 3-Peptide Mix

Results

Pi^3 Reagent:

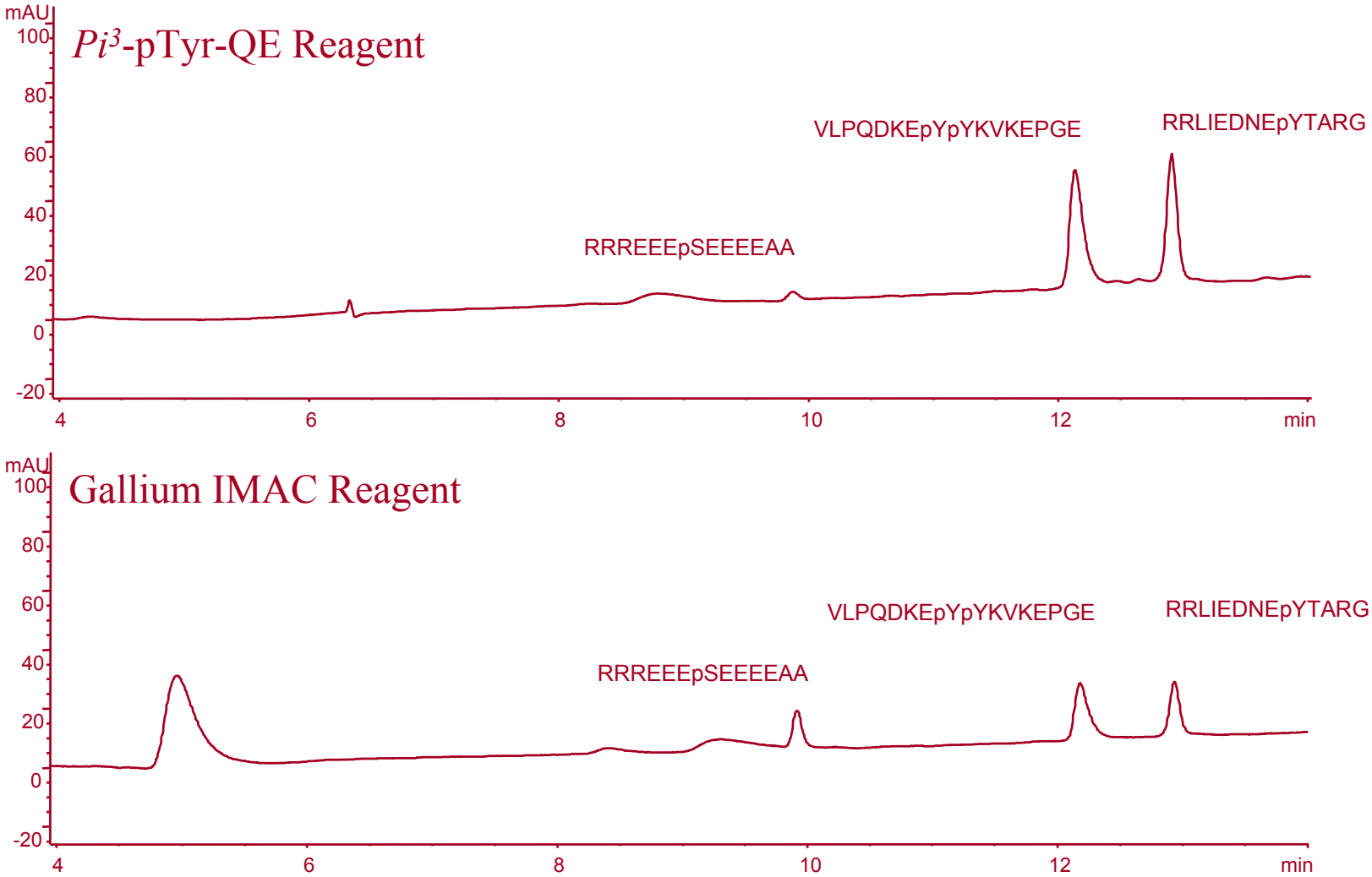
- VLPQDKEpYpYKVKEPGE recovery 90%
- RRLIEDNEpYTARG recovery 81%
- 8.5 mol% non-specific binding of RREEETEEE

IMAC Reagent:

- VLPQDKEpYpYKVKEPGE recovery 29%
- RRLIEDNEpYTARG recovery 27%
- 25 mol% non-specific binding of RREEETEEE

Phosphotyrosine Peptide-Specific Isolation

From 250 pmole Phosphotyrosine, Phosphoserine, Phosphothreonine 4-Peptide Mix



Phosphotyrosine Peptide-Specific Isolation

From 250 pmole Phosphotyrosine, Phosphoserine, Phosphothreonine 4-Peptide Mix

Results

Pi^3 Reagent:

- VLPQDKEpYpYKVKEPGE recovery 67%
- RRLIEDNEpYTARG recovery 65%
- RRREEEpSEEEEAA recovery 6%
- KRpTIRR recovery 0%

IMAC Reagent:

- VLPQDKEpYpYKVKEPGE recovery 30%
- RRLIEDNEpYTARG recovery 26%
- RRREEEpSEEEEAA recovery 38%
- KRpTIRR recovery 0%

Summary

- The Pi^3 Phosphotyrosine-QE reagent is a novel, non-water soluble reagent for the specific isolation of peptides, including phosphotyrosine and other phosphopeptides from complex peptide mixtures.
- The method works for peptide mixtures over a wide range of sample quantities.
- The method works for peptide mixtures containing low abundances of the desired peptide(s).
- Compared with a gallium IMAC reagent, *Pi*^{3}-pTyr-QE reagent provides substantially higher phosphopeptide yields, more reproducible affinity for the phosphopeptides and lower non-specific binding.