Triple Quadrupole Mass Spectromery-Based Peptide Assays Using Intelligent SRM (iSRM)

Reiko Kiyonami[1], Alan Schoen[1], Amol Prakash[1], Huy Nguyen[1], Scott Peterman[1], Vlad Zabrouskov[1], Andreas Huhmer[1], Sarah Robinson[1], Martin Hornshaw[1], Madalina Oppermann [1], Nathalie Selevsek[2], Bruno Domon[2]

[1] Thermo Fisher Scientific. [2] ETH Zurich, Switzerland

Introduction

The commonly used SRM technique provides sensitive and precise quantitative results by monitoring one or several primary SRM transitions per targeted compound. Recently this technique was extended to simultaneously confirm the identity and quantify multiple compounds in one HPLC/MS run by monitoring eight or more SRM transitions per compound. The bottleneck of this approach is that only a limited number of compounds can be targeted in one run because of the time required to monitor each transition. The newly developed instrument control software can use the specificy of a small subset of SRM transitions to simultaneously quantify and intelligently trigger the full list for confirmation, thereby allowing the analysis of up to 1000 compounds in a single LC-MS run.

Experimental

A triple quadrupole mass spectrometer equipped with a nanoLC pump and a nanospray source was used. The intelligent SRM method utilizes SRM specificity in two ways. The first is compound specific quantification using a time based SRM acquisition, which monitors several primary transitions for each compound. The second is a data dependent SRM acquisition, which monitors both those primary and additional secondary transitions and is triggered only when the intensities of all primary SRM transitions simultaneously exceed the defined intensity threshold. For large scale screening experiments, the dynamic exclusion was used to trigger secondary acquisition only once for each peak for providing sufficient structural information to confirm the compound's identity without perturbing the quantification obtained with the primary SRM list.

Results and discussion

The technique was applied to quantify precisely and confirm identity of peptides in complex biological samples, including yeast cell lysates, and pesticides in orange oil. By monitoring multiple transitions, it is possible to use very low intensity thresholds to accurately trigger the data dependent SRM scan. The trigger is the leading edge of the LC peak and relatively independent of the LC peak intensity. Using known LC peak widths, the strongest intensity point to obtain the data dependant scan is easily anticipated. Instead of a full product ion scan, high quality SRM spectra monitor eight or more transitions at that point. The resulting eight spectra are assembled into a pseudo full scan MS/MS spectra that can be used for confirming the identity of the peptides by matching them to reference spectra stored in a library. Using a constant cycle time allows enough points across the peaks from the primary SRM scans for precise quantification. With this approach, the instrument is able to confirm and quantify most targeted peptides in the low attomol range.