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Detection and Quantification of IL-6 and IL-11 in Human Serum

Introduction

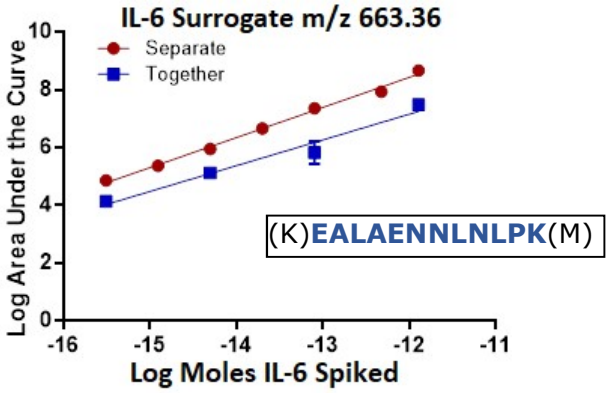
Accurate, sensitive methods (picomolar range and below) for detecting and quantifying circulating cytokines are desirable given their enormous clinical importance as bioregulators and potential biomarkers[1,2]. Recent improvements in liquid chromatography coupled to mass spectrometry (LC-MS) have garnered significant advantages relative to current techniques[4,5]. In particular, LC-MS bypasses concerns of pleiotropism and redundancy effects present in cellular assays, and being a direct measurement approach tends to reflect concentrations accurately[2,3].

Methods

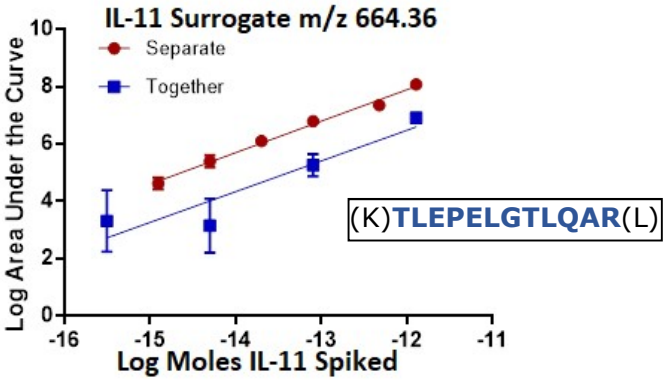
Recombinant human Interleukin 6 (IL-6) or Interleukin 11 (IL-11) (Peprotech) were spiked into human serum to achieve a final concentration of 0.31, 1.25, 5, 20, 80, 320, 1280 fmol/µL, applied either pre-treated with trypsin (*Separate*) or combined prior to trypsin treatment (*Together*). Samples were denatured, reduced, alkylated and digested with trypsin using standard protocols. Reversed phase chromatography was applied at 350nL/min for a linear gradient from 1% to 95% acetonitrile+0.02% formic acid over 60 minutes. Detection was accomplished using the Thermo Q-Exactive.

Results

The graphs below represent a single surrogate peptide selected from either IL-6 or IL-11 based on performance as a surrogate marker for the detection of the originating cytokine.



The response (area under the curve) increased linearly as the amount of cytokine spiked was increased from 300 attomoles to 1.28 µmoles. For all cytokine concentrations tested, we observed a lower response when cytokines and serum were treated concurrently (*Together*) that when they were treated separately (*Separate*) due most likely to matrix effects.



Conclusions

Spectrus scientists were able to detect and quantify low amounts of IL-6 and IL-11 in serum by monitoring surrogate peptides using nanoflow chromatograhy coupled to high resolution orbitrap mass spectrometry. We observed a linear response from 300 attomoles to 1.3 micromoles of protein on column. Advantages of LC-MS for this approach are shown here:

Sensitivity	Low abundance proteins as low as attomole amounts can be detected[6]
Specificity	Surrogate peptides analyzed are directly obtained from the cytokine being studied thus reducing the possibility of false positives
Multiplexing	Several cytokines can be studied simultaneously[7]
Signal Enhancement	Ability to work in tandem with other techniques, such as liquid chromatography and immunoprecipitation

These data demonstrate a robust method that can be applied generally to the detection of protein targets in low abundance in complex matrices.

References

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