CLAIMS:

1. A method for absolute quantification of a first polypeptide in a sample comprising a plurality of polypeptides comprising the first polypeptide and a second polypeptide,

wherein the first polypeptide is at least 10-fold lower in abundance than the second polypeptide,

the method comprising:

(a) analyzing peptide products of the plurality of polypeptides at a plurality of sample loading quantities using a liquid chromatography/mass spectrometry (LC/MS) technique to obtain MS signals of ions of the peptide products of the plurality of polypeptides at each of the plurality of sample loading quantities,

wherein the plurality of sample loading quantities comprises a first sample loading quantity and a second sample loading quantity, and

wherein the first sample loading quantity is greater than the second sample loading quantity;

(b) calculating the average or sum of an MS signal for:

(i) a top set of n number of qualified ions of peptide products of the first polypeptide with the highest MS signals at the first sample loading quantity (A);

(ii) a top set of n number of qualified ions of peptide products of the second polypeptide with the highest MS signals at the second sample loading quantity (B);

(iii) a middle set of m number of qualified ions of peptide products of the second polypeptide at the first sample loading quantity (C),

wherein the middle set of qualified ions of the peptide products of the second polypeptide has a lower quantification error than the top set of qualified ions of the peptide products of the second polypeptide for the first sample loading quantity and the second sample loading quantity; and

(iv) the middle set of qualified ions of peptide products of the second polypeptide at the second sample loading quantity (D),

wherein A, B, C, and D are all calculated using the average or all calculated using the sum of the MS signal; and

(c) determining an absolute quantity of the first polypeptide in the first sample loading quantity based on the following formula:

[(*A*)/(*C*)]*(mole of the second polypeptide at the first loading quantity)*[(*D*)/(*B*)],

or mathematical equivalents thereof.

2. A method for absolute quantification of a first polypeptide in a sample comprising a plurality of polypeptides comprising the first polypeptide and a second polypeptide,

wherein the first polypeptide is at least 10-fold lower in abundance than the second polypeptide, the method comprising:

(a) obtaining MS signals of ions of peptide products of the plurality of polypeptides,

wherein said MS signals of ions of the peptide products are obtained by analyzing the peptide products of the plurality of polypeptides using a liquid chromatography/mass spectrometry (LC/MS) technique,

wherein MS signals of the peptide products are obtained for each of a plurality of sample loading quantities comprising a first sample loading quantity and a second sample loading quantity, and

wherein the first sample loading quantity is greater than the second sample loading quantity;

(b) calculating the average or sum of an MS signal for:

(i) a top set of n number of qualified ions of peptide products of the first polypeptide with the highest MS signals at the first sample loading quantity (A);

(ii) a top set of n number of qualified ions of peptide products of the second polypeptide with the highest MS signals at the second sample loading quantity (B);

(iii) a middle set of m number of qualified ions of peptide products of the second polypeptide at the first sample loading quantity (C),

wherein the middle set of qualified ions of the peptide products of the second polypeptide has a lower quantification error than the top set of qualified ions of the peptide products of the second polypeptide for the first sample loading quantity and the second sample loading quantity; and

(iv) the middle set of qualified ions of peptide products of the second polypeptide at the second sample loading quantity (D),

wherein A, B, C, and D are all calculated using the average or all calculated using the sum of the MS signal; and

(c) determining an absolute quantity of the first polypeptide in the first sample loading quantity based on the following formula:

[(*A*)/(*C*)]*(mole of the second polypeptide at the first loading quantity)*[(*D*)/(*B*)],

or mathematical equivalents thereof.

3. The method of claim 2, wherein the middle set of qualified ions of peptide products of the second polypeptide is selected based on quantification error of the qualified ions of peptide products of the second polypeptide from (1) the plurality of sample loading quantities, or (2) the first sample loading quantity and/or the second sample loading quantity.

4. The method of claim 3, further comprising selecting the middle set of qualified ions of peptide products of the second polypeptide.

5. The method of claim 2, wherein each of the top set of qualified ions of peptide products is different than each of the middle set of qualified ions.

6. The method of claim 2, wherein the MS signal is ionization intensity, peak height, peak area, or peak volume.

7. The method of claim 2, wherein the LC/MS technique further comprises one or more the following:

   (a) identifying the peptide products by amino acid sequence;

   (b) identifying the first polypeptide by a protein identifier; and

   (c) identifying one or more of the plurality of polypeptides by a protein identifier.

8. The method of claim 2, further comprising determining the absolute quantity of the second polypeptide.

9. The method of claim 2, wherein the first polypeptide is a host cell protein and/or a biomarker.

10. The method of claim 2, wherein the first polypeptide is at least 100-fold lower in abundance than the second polypeptide.

11. The method of claim 2, wherein the second polypeptide is a recombinant polypeptide produced by a host cell and/or a therapeutic polypeptide.

12. The method of claim 2, wherein the sample is a cell culture sample, a blood sample, or a serum sample.

13. The method of claim 12, wherein the sample is a blood sample or serum sample, and wherein the second polypeptide is serum albumin.

14. The method of claim 2, wherein the sample is a pharmaceutical product or an intermediate thereof.

15. The method of claim 2, further comprising selecting the second sample loading quantity based on MS signals of the second set of peptide products and/or selecting the first sample loading quantity based on MS signals of the first set of peptide products.

16. The method of claim 2, wherein n and/or m is 3.

17. The method of claim 2, wherein the middle set of qualified ions of peptide products of the second polypeptide are selected based on the sequences of each of the peptide products.

18. A method for detecting a contaminate polypeptide in the production of a therapeutic polypeptide, the method comprising:

(a) obtaining a sample comprising the therapeutic polypeptide;

(b) determining if the contaminate polypeptide is present in the sample;

wherein the presence of the contaminate polypeptide is based on the absolute quantification of the contaminate polypeptide in the sample using the method of claim 2.