a peptide) is determined based on the MS responses (e.g., peak areas) of the modified peptide and the unmodified peptide with the following assumptions: (1) unmodified and modified peptides have reproducible recovery between labs; (2) unmodified and modified peptides have the same MS response factor, and (3) artificially induced attribute changes are negligible.

[0008] Due to these assumptions, a conventional MAM procedure depends on multiple required conditions, including that (1) the digestion efficiency is reproducible between labs; (2) the MS instrument conditions are exactly the same; and (3) the sample preparations performed by different labs introduce minimal or a constant amount of artificial modifications. In reality, however, these required conditions are difficult to meet because of variations of sample preparation procedures, analyst habits, instruments, reagent quality, etc. Peptide recovery can also fluctuate, causing additional variability.

[0009] Additional challenges of meeting the required conditions include differences in MS instrument model or instrument settings. For example, the manner in which one lab instrument is maintained can differ from the manner in which a second lab instrument lab is maintained. In addition, a response factor for peptides containing different variants across different labs may differ. Further challenges of meeting the required conditions include variations of sample preparation procedure, analyst habit, device and reagent quality, as well as instrument condition. In addition, the amount of artificially introduced modification may vary.

[0010] As a result, conventional MS-based MAM methods lack robustness in lab-to-lab and/or instrument-to-instrument variability.

[0011] In addition, a major challenge of mass spectrometry-based multi-attribute method (MAM) is its high variability between analysts and instruments. For reproducible attribute measurements, not only a similar instrument model is required for all analytical labs, the instrument must also be tuned to the same condition. This poses great long-term challenges, considering the rapid development of new chromatography and mass spectrometry technologies. In addition, difference in digestion efficiency and artificial modifications (e.g., oxidation, deamidation, Asp-isomerization, and fragmentation) during sample preparation also contribute to variability between laboratories. These challenges must be resolved to ensure a long-term success of MAM, e.g., in a cGMP environment.

BRIEF SUMMARY

[0011a] According to a first aspect of the present invention, there is provided a calibration system configured to reduce lab-to-lab or instrument-to-instrument variability of Multi-Attribute Methods (MAM) via run-time signal intensity calibration, the calibration system comprising:

a first MAM-based instrument including a first detector, the first MAM-based instrument having a first instrument condition defined by at least one of: (1) a first instrument model or (2) a first set of settings, the first MAM-based instrument configured to receive a first sample and a reference standard, and the first MAM-based instrument further configured, via the first detector, to detect a first sample isoform from the first sample and a first reference standard isoform from the reference standard;

one or more processors associated with the first MAM-based instrument, the one or more processors associated with the first MAM-based instrument configured to determine, via a first MAM iteration, a first set of correction factors corresponding to the first sample isoform, wherein the first set of correction factors is based on the reference standard, and the one or more processors associated with the first MAM-based instrument further configured to determine a first set of sample abundance values corresponding to the first sample isoform, wherein the first set of sample abundance values is based on the first set of correction factors;

a second MAM-based instrument including a second detector, the second MAM-based instrument having a second instrument condition defined by at least one of: (1) a second instrument model or (2) a second set of settings,

wherein the second instrument condition differs from the first instrument condition,

the second MAM-based instrument configured to receive a second sample and the reference standard, and the second MAM-based instrument further configured to, via the second detector, detect a second sample isoform from the second sample and a second reference standard isoform from the reference standard; and

one or more processors associated with the second MAM-based instrument, the one or more processors associated with the second MAM-based instrument configured to determine, via a second MAM iteration, a second set of correction factors corresponding to the second sample isoform, wherein the second set of correction factors is based on the reference standard, and the one or more processors associated with the second MAM-based instrument further configured to determine a second set of sample abundance values corresponding to the second sample isoform,

wherein the second set of sample abundance values is based on the second set of correction factors, and

wherein a variance value of the first set of sample abundance values and the second set of sample abundance values is reduced based on the first set of correction factors and the second set of correction factors.

[0011b] According to a second aspect of the present invention, there is provided a calibration method for reducing lab-to-lab or instrument-to-instrument variability of Multi-Attribute Methods (MAM) via run-time signal intensity calibration, the calibration method comprising:

receiving, at a first MAM-based instrument including a first detector, a first sample and a reference standard;

detecting, via the first detector, a first sample isoform from the first sample and a first reference standard isoform from the reference standard;

determining, via one or more processors associated with the first MAM-based instrument for a first MAM iteration, a first set of correction factors corresponding to the first sample isoform, wherein the first set of correction factors is based on the reference standard;

determining, via the one or more processors associated with the first MAM-based instrument, a first set of sample abundance values corresponding to the first sample isoform, wherein the first set of sample abundance values is based on the first set of correction factors, and wherein the first MAM-based instrument includes a first instrument condition defined by at least one of: (1) a first instrument model or (2) a first set of settings;

receiving, at a second MAM-based instrument including a second detector, a second sample and the reference standard;

detecting, via the second detector, a second sample isoform from the second sample and a second reference standard isoform from the reference standard;

determining, via one or more processors associated with the second MAM-based instrument for a second MAM iteration, a second set of correction factors corresponding to the second sample isoform, wherein the second set of correction factors is based on the reference standard;

determining, via the one or more processors associated with the second MAM-based instrument, a second set of sample abundance values corresponding to the second sample

isoform, wherein the second set of sample abundance values is based on the second set of correction factors, and wherein the second MAM-based instrument includes a second instrument condition defined by at least one of: (1) a second instrument model or (2) a second set of settings,

wherein the second instrument condition differs from the first instrument condition, and

wherein a variance value of the first set of sample abundance values and the second set of sample abundance values is reduced based on the first set of correction factors and the second set of correction factors.

[0011c] According to a third aspect of the present invention, there is provided a calibration method for reducing variability of a MAM-based instrument for multiple time periods via run-time signal intensity calibration, the calibration method comprising:

receiving, at a MAM-based instrument for a first time period, a first sample and a reference standard;

detecting, via a detector of the MAM-based instrument for the first time period, a first sample isoform from the first sample and a first reference standard isoform from the reference standard;

determining, via one or more processors for a first MAM iteration for the first time period, a first set of correction factors corresponding to the first sample isoform, wherein the first set of correction factors is based on the reference standard;

determining, via the one or more processors via the first MAM iteration for the first time period, a first set of sample abundance values corresponding to the first sample isoform, wherein the first set of sample abundance values is based on the first set of correction factors, and wherein the MAM-based instrument, for the first time period, includes a first instrument condition defined by a first set of settings;

receiving, at the MAM-based instrument for a second time period, a second sample and the reference standard;

detecting, via the detector of the MAM-based instrument for the second time period, a second sample isoform from the second sample and a second reference standard isoform from the reference standard;

determining, via the one or more processors for a second MAM iteration for the second time period, a second set of correction factors corresponding to the second sample isoform, wherein the second set of correction factors is based on the reference standard; and

determining, via the one or more processors via the second MAM iteration for the second time period, a second set of sample abundance values corresponding to the second sample isoform, wherein the second set of sample abundance values is based on the second set of correction factors, and wherein the MAM-based instrument, for the second time period, includes a second instrument condition defined by a second set of settings,

wherein the second instrument condition differs from the first instrument condition, and

wherein a variance value of the first set of sample abundance values and the second set of sample abundance values is reduced based on the first set of correction factors and the second set of correction factors.

[0011d] According to a fourth aspect of the present invention, there is provided a calibration system configured to reduce lab-to-lab or instrument-to-instrument variability of Multi-Attribute Methods (MAM) via run-time signal intensity calibration, the calibration system comprising:

a first MAM-based instrument including a first detector, the first MAM-based instrument having a first instrument condition defined by at least one of: (1) a first instrument model or (2) a first set of settings, the first MAM-based instrument configured to receive a first sample and a reference standard, and the first MAM-based instrument further configured, via the first detector, to detect a first sample isoform from the first sample and a first reference standard isoform from the reference standard, wherein the first sample has a first preparation type;

one or more processors associated with the first MAM-based instrument, the one or more processors associated with the first MAM-based instrument configured to determine, via a first MAM iteration, a first set of correction factors corresponding to the first sample isoform, wherein the first set of correction factors is based on the reference standard, and the one or more processors associated with the first MAM-based instrument further configured to determine a first set of sample abundance values corresponding to the first sample isoform, wherein the first set of sample abundance values is based on the first set of correction factors;

a second MAM-based instrument including a second detector, the second MAM-based instrument having a second instrument condition defined by at least one of: (1) a second instrument model or (2) a second set of settings,

wherein the second instrument condition differs from the first instrument condition,

the second MAM-based instrument configured to receive a second sample and the reference standard, and the second MAM-based instrument further configured to, via the second detector, detect a second sample isoform from the second sample and a second reference standard isoform from the reference standard, wherein the second sample has a second preparation type, and

wherein the first preparation type differs from the second preparation type causing a variance between the first sample and the second sample, the variance caused by an artificial change of attribute abundance during sample preparation; and

one or more processors associated with the second MAM-based instrument, the one or more processors associated with the second MAM-based instrument configured to determine, via a second MAM iteration, a second set of correction factors corresponding to the second sample isoform, wherein the second set of correction factors is based on the reference standard, and the one or more processors associated with the second MAM-based instrument further configured to determine a second set of sample abundance values corresponding to the second sample isoform, wherein the second set of sample abundance values is based on the second set of correction factors, and

wherein a variance value of the first set of sample abundance values and the second set of sample abundance values is reduced based on the first set of correction factors and the second set of correction factors,

wherein at least one of (1) the one or more processors associated with the first MAM-based instrument, via the first MAM iteration, or (2) the one or more processors associated with the second MAM-based instrument, via the second MAM iteration, determines a quality attribute to reduce the variance between the first sample and the second sample, the quality attribute associated with a type-2 attribute or a type-3 attribute.

[0011e] According to a fifth aspect of the present invention, there is provided a calibration method for reducing lab-to-lab or instrument-to-instrument variability of Multi-Attribute Methods (MAM) via run-time signal intensity calibration, the calibration method comprising:

receiving, at a first MAM-based instrument, a first sample and a reference standard, the first MAM-based instrument including a first detector, and the first MAM-based instrument having a first instrument condition defined by at least one of: (1) a first instrument model or (2) a first set of settings;

detecting, by the first detector of the first MAM-based instrument, a first sample isoform from the first sample and a first reference standard isoform from the reference standard, wherein the first sample has a first preparation type;

determining, by one or more processors associated with the first MAM-based instrument, via a first MAM iteration, a first set of correction factors corresponding to the first sample isoform, wherein the first set of correction factors is based on the reference standard;

determining, by the one or more processors associated with the first MAM-based instrument, a first set of sample abundance values corresponding to the first sample isoform, wherein the first set of sample abundance values is based on the first set of correction factors;

receiving, by a second MAM-based instrument, a second sample and the reference standard, the second MAM-based instrument including a second detector, the second MAM-based instrument having a second instrument condition defined by at least one of: (1) a second instrument model or (2) a second set of settings, wherein the second instrument condition differs from the first instrument condition;

detecting, by the second detector of the second MAM-based instrument, a second sample isoform from the second sample and a second reference standard isoform from the reference standard, wherein the second sample has a second preparation type, and wherein the first preparation type differs from the second preparation type causing a variance between the first sample and the second sample, the variance caused by an artificial change of attribute abundance during sample preparation;

determining, by one or more processors associated with the second MAM-based instrument, via a second MAM iteration, a second set of correction factors corresponding to the

second sample isoform, wherein the second set of correction factors is based on the reference standard; and

determining, by the one or more processors associated with the second MAM-based instrument, a second set of sample abundance values corresponding to the second sample isoform, wherein the second set of sample abundance values is based on the second set of correction factors,

wherein a variance value of the first set of sample abundance values and the second set of sample abundance values is reduced based on the first set of correction factors and the second set of correction factors, and

wherein at least one of (1) the one or more processors associated with the first MAM-based instrument, via the first MAM iteration, or (2) the one or more processors associated with the second MAM-based instrument, via the second MAM iteration, determines a quality attribute to reduce the variance between the first sample and the second sample, the quality attribute associated with a type-2 attribute or a type-3 attribute.

[0011f] According to a sixth aspect of the present invention, there is provided a calibration system configured to reduce lab-to-lab or instrument-to-instrument variability of Multi-Attribute Methods (MAM) via run-time signal intensity calibration, the calibration system comprising:

a first MAM-based instrument including a first detector, the first MAM-based instrument having a first instrument condition defined by at least one of: (1) a first instrument model or (2) a first set of settings, the first MAM-based instrument configured to receive a first sample, a reference standard, and a stressed standard, and the first MAM-based instrument further configured, via the first detector, to detect a first sample isoform from the first sample, a first reference standard isoform from the reference standard, and a stressed reference standard isoform from the stressed standard;

one or more processors associated with the first MAM-based instrument, the one or more processors associated with the first MAM-based instrument configured to determine, via a first MAM iteration, a first set of correction factors corresponding to the first sample isoform, wherein the first set of correction factors is based on the reference standard and the stressed standard, and the one or more processors associated with the first MAM-based instrument further configured to determine a first set of sample abundance values corresponding to the first sample

isoform, wherein the first set of sample abundance values is based on the first set of correction factors;

a second MAM-based instrument including a second detector, the second MAM-based instrument having a second instrument condition defined by at least one of: (1) a second instrument model or (2) a second set of settings,

wherein the second instrument condition differs from the first instrument condition,

the second MAM-based instrument configured to receive a second sample, the reference standard, and the stressed standard, and the second MAM-based instrument further configured to, via the second detector, detect a second sample isoform from the second sample, a second reference standard isoform from the reference standard, and a second stressed standard isoform from the stressed standard; and

one or more processors associated with the second MAM-based instrument, the one or more processors associated with the second MAM-based instrument configured to determine, via a second MAM iteration, a second set of correction factors corresponding to the second sample isoform, wherein the second set of correction factors is based on the reference standard and the stressed standard, and the one or more processors associated with the second MAM-based instrument further configured to determine a second set of sample abundance values corresponding to the second sample isoform, wherein the second set of sample abundance values is based on the second set of correction factors, and

wherein a variance value of the first set of sample abundance values and the second set of sample abundance values is reduced based on the first set of correction factors and the second set of correction factors.

[0011g] According to a seventh aspect of the present invention, there is provided a calibration method for reducing lab-to-lab or instrument-to-instrument variability of Multi-Attribute Methods (MAM) via run-time signal intensity calibration, the calibration method comprising:

receiving, at a first MAM-based instrument, a first sample, a reference standard, and a stressed standard, the first MAM-based instrument including a first detector, and the first MAM-based instrument having a first instrument condition defined by at least one of: (1) a first instrument model or (2) a first set of settings;

detecting, by the first detector of the first MAM-based instrument, a first sample isoform from the first sample, a first reference standard isoform from the reference standard, and a stressed reference standard isoform from the stressed standard;

determining, by one or more processors associated with the first MAM-based instrument, via a first MAM iteration, a first set of correction factors corresponding to the first sample isoform, wherein the first set of correction factors is based on the reference standard and the stressed standard;

determining, by the one or more processors associated with the first MAM-based instrument, a first set of sample abundance values corresponding to the first sample isoform, wherein the first set of sample abundance values is based on the first set of correction factors;

receiving, at a second MAM-based instrument, a second sample, the reference standard, and the stressed standard, the second MAM-based instrument including a second detector, and the second MAM-based instrument having a second instrument condition defined by at least one of: (1) a second instrument model or (2) a second set of settings, wherein the second instrument condition differs from the first instrument condition;

detecting, by the second detector of the second MAM-based instrument, a second sample isoform from the second sample, a second reference standard isoform from the reference standard, and a second stressed standard isoform from the stressed standard;

determining, by one or more processors associated with the second MAM-based instrument, via a second MAM iteration, a second set of correction factors corresponding to the second sample isoform, wherein the second set of correction factors is based on the reference standard and the stressed standard; and

determining, by the one or more processors associated with the second MAM-based instrument, a second set of sample abundance values corresponding to the second sample isoform, wherein the second set of sample abundance values is based on the second set of correction factors,

wherein a variance value of the first set of sample abundance values and the second set of sample abundance values is reduced based on the first set of correction factors and the second set of correction factors.