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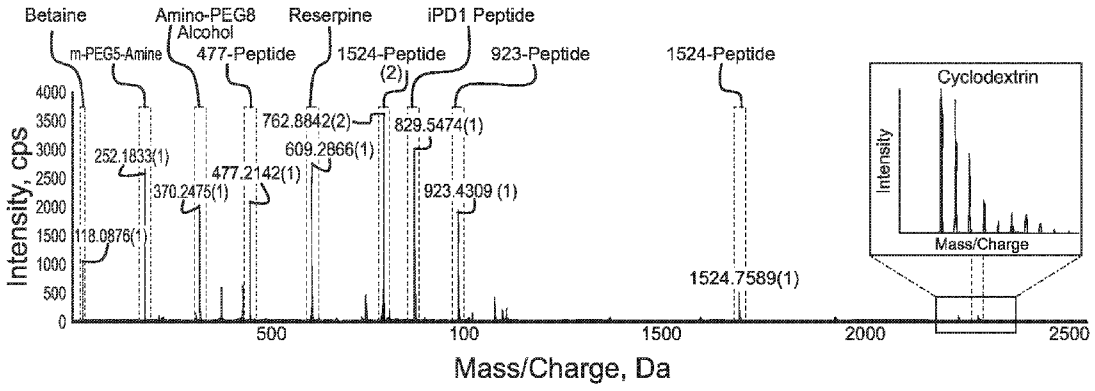


FIG. 6A

(57) Abstract: Calibrant compositions comprising a plurality of synthetic peptides for calibration of a mass spectrometer in either positive or negative mode are provided. The synthetic peptide calibrants exhibit desirable ionization characteristics, solubility, and solution stability, and are easily washed out of the system such that no interfering signals are left behind. Methods for calibration of mass spectrometer in positive or negative ion mode using a single calibrant composition are also provided.

WO 2024/257055 A2

USE OF CUSTOM-DESIGNED PEPTIDES FOR MASS CALIBRATION

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 63/508,339, filed June 15, 2023, the disclosure of which is hereby incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING

The instant application contains a Sequence Listing, which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML file, created on June 14, 2024, is named Sequence-Listing-18768-0086WOU1.xml and is 9,909 bytes in size.

BACKGROUND

Calibration and tuning of mass spectrometers are important to ensure mass accuracy. It is also important to assure that the instrument is in good working order prior to sample analysis. Well-defined standards are required to achieve the above goals.

Several issues with older generation mass spectrometry calibrators have been identified. (U.S. Pat. No. 9257267). A few older generation calibrator components can suffer from stability issues and give rise to extra interfering signals, for example, while sitting on the calibration delivery system (CDS) of the mass spectrometer instrument. In addition, some of the high mass components such as phosphazene compounds can suffer from "stickiness," which means they can be very difficult to flush out of the system. These sticky signals from the calibrants could interfere with the masses of the targeted analytes giving rise to complicated and misleading spectral data. Current calibration mixtures typically employ two separate solutions for positive and negative calibrants. In addition, negative calibration in older generation calibrants is typically achieved by a series of adduct ions leading to serious reliability and variability issues.

In order to address the above-mentioned shortcomings, it is desirable to provide a group of custom-designed peptide molecules with tailored properties to be used as effective calibrants. One advantage is that a single peptide can be used as both positive and negative calibrants, which is amenable to a single bottle formulation to cover both positive and negative masses. Moreover, the dependency on adduct ions for negative masses can be eliminated when custom peptides are used. It is desirable to provide synthetic peptides that can be easily washed out of the system such that no interfering signals are left behind, unlike widely used phosphazenes.

SUMMARY

A calibrant composition is provided for calibration of a mass spectrometer in either positive or negative mode, the composition comprising a plurality of synthetic peptides each comprising: a molecular weight in a range of 220 g/mol to 2200 g/mol and at least one amino acid residue comprising an aromatic side chain. In some cases, the aromatic side chain is selected from the group consisting of phenylalanine and tryptophan.

The calibrant composition may include two or more, three or more, or four or more of the synthetic peptides. In some cases, the synthetic peptides are soluble in an aqueous buffer in at least 1 mg/mL, or at least 2 mg/mL at 4 deg C.

The calibrant composition may include synthetic peptides each comprising from 3 to 23 naturally occurring amino acid residues or unnatural amino acid residues. The naturally occurring amino acid residues may be selected from the group consisting of alanine, asparagine, glutamine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, and valine.

The calibrant composition may include synthetic peptides each comprising at least one amino acid residue selected from the group consisting of serine and threonine.

The calibrant composition may include synthetic peptides each comprising at least one amino acid residue selected from the group consisting of asparagine and glutamine.

The calibrant composition may include a plurality of synthetic peptides, wherein the synthetic peptides each do not include an amino acid residue selected from the group consisting of arginine, lysine, aspartic acid, cysteine, glutamic acid, histidine, methionine, proline, and tyrosine.

The calibrant composition may include a plurality of synthetic peptides, wherein each of the synthetic peptides does not include a chargeable amino acid residue. In some cases, the synthetic peptides do not include a lysine, arginine, histidine, aspartic acid, or glutamic acid residue.

The calibrant composition may include a plurality of synthetic peptides, wherein the plurality of synthetic peptides comprises two or more, two to six, or three to five of the synthetic peptides.

In some embodiments, the synthetic peptides may be selected from the group consisting of 380-peptide (SEQ ID NO: 1); 477-peptide (SEQ ID NO: 2); 618-peptide (SEQ ID NO: 3); 823-peptide (SEQ ID NO: 4); 833-peptide (SEQ ID NO: 5); 919-peptide (SEQ ID NO: 6); 923-peptide (SEQ ID NO: 7); 1520-peptide (SEQ ID NO: 8); 1522-peptide (SEQ ID NO: 9); 1524-peptide (SEQ ID NO: 10); and 2219-peptide (SEQ ID NO: 11).

The calibrant composition may include a plurality of synthetic peptides and further comprise one or more organic calibrant compounds. In some cases, the one or more organic calibrant compounds may comprise a discrete length polyethylene glycol amine terminated with a C_{1-6} alkyl or a hydroxy group. These types of compounds comprise a polyethylene glycol compound having a defined chain length and molecular weight, unlike conventional polyethylene glycol compounds that comprise a number of compounds having a range of molecular weights. In some cases, the discrete length polyethylene glycol amine has a molecular weight in a range from 105 g/mol to 692 g/mol.

The calibrant composition may include a plurality of synthetic peptides, and further comprise a naturally occurring peptide, protein, or fragment thereof. In some

cases, the naturally occurring peptide, protein, or fragment thereof may be selected from the group consisting of iPD1 peptide, des-Arg^1-bradykinin, angiotensin I, angiotensin II, substance P, bombesin, Glu^1-fibrinopeptide B, ACTH (1-17 clip), ACTH (18-39 clip), ACTH (7-38 clip), somatostatin, neurotensin, and renin.

In some cases, the calibrant composition may include one or more custom made oligonucleotides.

In some cases, the calibrant composition does not comprise a phosphazene compound.

The calibrant composition may be in a liquid form comprising an aqueous organic solvent system.

The aqueous organic solvent system may comprise water and a water-miscible organic solvent in a range of 99:1 to 1:99 v/v optionally wherein the water-miscible organic solvent is selected from the group consisting of acetonitrile, methanol, isopropanol, ethanol, and n-propanol, optionally wherein the solvent system comprises a mixture of water and acetonitrile. The solvent system may comprise a mobile phase modifier. The modifier may be selected from the group consisting of formic acid, ammonium formate, ammonium acetate, triethylamine, trifluoroacetic acid, and difluoroacetic acid, and hexafluoroisopropanol.

The calibrant composition may be in a liquid form in a ready-to-use concentration. In some cases, the calibrant composition in ready-to-use concentration comprises 0.1-10 uM, or 1-4 uM of each of the one or more synthetic peptides.

A calibrant composition is provided for calibration of a mass spectrometer in either positive or negative mode, comprising a plurality of synthetic peptides; one or more discrete length polyethylene glycol amines; one or more of the organic calibrant compounds; a naturally occurring peptide, protein, or fragment thereof; and a solvent system.

The calibrant composition comprising a plurality of synthetic peptides can be stable for at least 3 months at 25 deg C when stored away from light as determined for each of the one or more synthetic peptides by mass spectrometry (MS), liquid chromatography-mass spectrometry (LC-MS), LC-MS/MS, or 100% ±20% starting peak area by high performance liquid chromatography (HPLC).

A method is provided for calibrating a mass spectrometer comprising: obtaining a mass spectrum of the calibrant composition of the disclosure; determining the differences between the expected mass peaks for each of the plurality of the synthetic peptides and the corresponding actual mass peaks obtained; and adjusting the mass spectrometer based on the differences between the expected and actual mass peaks.

A method is provided for calibration of a mass spectrometer, comprising providing a calibrant composition comprising a plurality of synthetic peptides, wherein the calibrant composition is suitable for use in either positive or negative ionization mode.

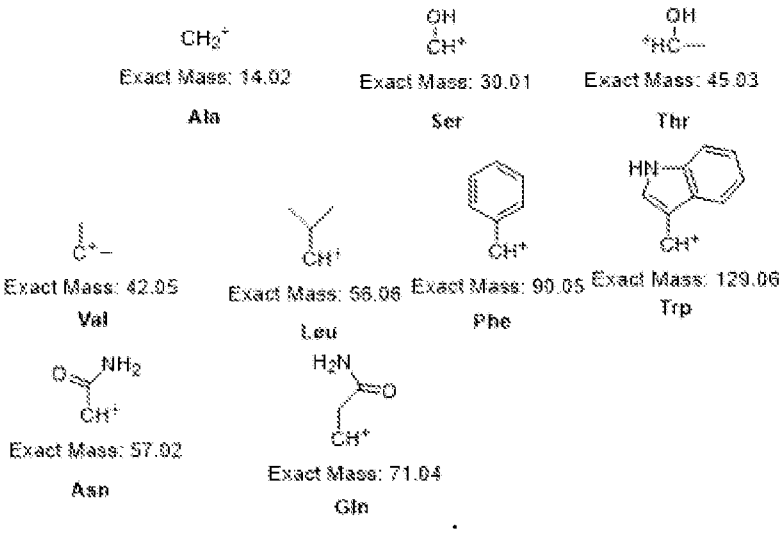
A method is provided for calibration of a mass spectrometer in either positive or negative mode, comprising providing a calibrant composition comprising a plurality of synthetic peptides, wherein the mass spectrometer is an electrospray ionization (ESI) mass spectrometer, an atmospheric pressure chemical ionization (APCI) mass spectrometer, fast atom bombardment (FAB) mass spectrometer, or a matrix-assisted laser desorption ionization (MALDI) mass spectrometer. In some cases, the method is performed in MS/MS mode.

A method is provided for calibration of a mass spectrometer, comprising providing a calibrant composition comprising a plurality of synthetic peptides, wherein the calibrant composition calibrates the mass spectrometer across a range of from 100 to 2200 Da or 50 to 1500 Da.

A kit is provided comprising in a container, a calibrant composition comprising a plurality of synthetic peptides in a solvent system; and instructions for use.

In some embodiments, the disclosure provides a method of providing a synthetic peptide calibrant, comprising selecting a peptide calibrant target mass in a range between 200 Da and 2,200 Da; selecting a poly-glycine peptide framework having a mass less than the target mass and comprising between 3 and 23 glycine residues; replacing the alpha proton in one or more, two or more, three or more, or four or more of the glycine residues with an amino acid side chain independently selected from a naturally occurring alpha-amino acid side chain or unnatural amino acid side chain to designate the synthetic peptide calibrant having the target mass; and synthesizing the designated synthetic peptide calibrant having the target mass.

In some cases, the naturally occurring alpha-amino acid side chains are independently selected from the group consisting of



In some embodiments, the synthetic peptide calibrant comprises at least one amino acid residue comprising an aromatic side chain, selected from the group consisting of phenylalanine and tryptophan.

In some embodiments, the synthetic peptide calibrant comprises at least one amino acid residue selected from the group consisting of serine and threonine. In some embodiments, the synthetic peptide calibrant comprises at least one amino acid residue selected from the group consisting of asparagine and glutamine.

In some embodiments, the synthetic peptide calibrant is soluble in an aqueous buffer in at least 1 mg/mL, or at least 2 mg/mL at 4 deg C.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows two representative glycine frameworks used for designing the synthetic peptides of the disclosure with appropriate properties.

FIG. 2 shows a list for selecting the appropriate side chain masses from selected natural amino acids.

FIG. 3 shows table 1 with properties of representative synthetic peptides according to the disclosure which were designed, synthesized, and evaluated by mass spectrometry. Table 1 shows the synthetic peptide ID, amino acid sequence, molecular weight and mass spec m/z data in positive and negative ion modes.

FIG. 4A shows positive ion mode mass spectrometry data for a selected panel of seven synthetic peptides from table 1 in different dilutions 1X (1uM, +TOF-MS, 100-2000 Da, upper panel), 3X (middle panel), and 5X (lower panel).

FIG. 4B shows negative ion mode mass spectrometry data for a selected panel of seven synthetic peptides from table 1 in different dilutions 1X (1uM, -TOF-MS, 100-2000 Da, upper panel), 3X (middle panel), and 5X (lower panel).

FIG. 5 shows table 2 listing components of representative calibration composition 2 including three synthetic peptides according to the disclosure 477-peptide (SEQ ID NO: 2), 923-peptide (SEQ ID NO: 7), and 1524-peptide (SEQ ID NO: 10).

FIG. 6A shows positive ion mode mass spectrometry spectrum from the formulation of table 2 (+TOF-MS, 100-2300 Da) including three synthetic peptides 477-peptide (SEQ ID NO: 2), 923-peptide (SEQ ID NO: 7), and 1524-peptide (SEQ ID NO: 10). The inset shows spectrum from formulation component triacetyl-beta-cyclodextrin (+TOF-MS, 2034-2044Da).

FIG. 6B shows negative ion mode mass spectrometry spectrum from the formulation of table 2 (-TOF-MS, 100-2300 Da) including three synthetic peptides 477-peptide (SEQ ID NO: 2), 923-peptide (SEQ ID NO: 7), and 1524-peptide (SEQ ID NO: 10). The inset shows spectrum from formulation component triacetyl-beta-cyclodextrin (-TOF-MS, 2060-2066Da).

FIG. 7 shows table 3 listing calibrant compounds of representative calibration composition 3 including four synthetic peptides according to the disclosure including 477-peptide (SEQ ID NO: 2), 618-peptide (SEQ ID NO: 3); 923-peptide (SEQ ID NO: 7); and 1524-peptide (SEQ ID NO: 10).

FIG. 8 shows a negative ion mass spectrum (-TOF-MS) in a range of 100-2200 from 0.511 to 0.921 min of calibrant composition 3, formula B. The inset table shows m/z and sum intensities for the calibrant composition.

DETAILED DESCRIPTION OF THE INVENTION

Finding the right compounds to be used as mass spectrometry calibrators can be a challenge, especially for the higher masses above 1000. Currently, most of the widely used high mass calibrators suffer from many drawbacks including prolonged sticking to the system and toxicity. Typical mass spectrometer calibration compositions use "sticky" phosphazenes for achieving high mass calibration. Molecules like phosphazenes can form multiple adducts with acid molecules, halide ions, and the like.

The synthetic peptides provided herein address a majority of those issues and can be sourced in high purity from multiple vendors. These synthetic peptides can be easily washed out of the system unlike molecules like phosphazenes. One single peptide can be used for both positive and negative mass calibrations, and that substantially helps to reduce the number of components required in the mixture. The presence of UV chromophores in the synthetic peptides helps to determine the concentration of these compounds accurately using UV and that helps the consistent manufacturing of the calibrant solutions in large scale.

Synthetic peptides provided herein have been tailor-made with desired properties to replace phosphazenes in calibrator compositions. The synthetic peptides provided herein can be easily washed away from the system and the same molecule can be used for both positive and negative calibration.

Currently, organic calibrant compounds like reserpine with limited ions are used for tandem MS2 and MS3 hardware calibration. Synthetic peptides provided herein are better alternatives for this task in part due to the availability of multitude of stronger 'b' and 'y' ions. By using the synthetic peptides there is no need to rely upon variable adduct-ions for negative calibration.

Synthetic Peptides

Synthetic peptides are provided herein that are suitable for use as mass calibrators for mass spectrometer calibration and tuning solutions.

The synthetic peptides comprise a target molecular weight to satisfy the desired mass range and spacing requirements. The synthetic peptides may each have a molecular weight in a range of about 220 g/mol to about 2,200 g/mol. In some cases, the synthetic peptides comprise from 3 to 23, 4 to 20, or 5 to 15 naturally occurring alpha-amino acid residues or unnatural amino acid residues.

The synthetic peptides comprise one or more, or two or more ultraviolet (UV) chromophore-containing aromatic amino acid residues. This feature allows orthogonal UV detection of the synthetic peptides to determine accurate concentrations of stock solutions in order to minimize manufacturing inconsistencies seen in certain in earlier versions of calibrators. The aromatic amino acid residues may be selected from the group consisting of phenylalanine and tryptophan.

The synthetic peptides of the present disclosure are designed to enhance solubility in aqueous solvent systems comprising a mixture of one or more miscible organic solvents and water. The synthetic peptides comprise a balance between hydrophobic and hydrophilic side chains to achieve the desired solubility.

The synthetic peptides may include one or more, two or more, three or more, or four or more hydrophilic amino acid residues that are devoid of chargeable side chains. The hydrophilic amino acid residues may be selected from Asparagine, Glutamine, Serine, and Threonine, and the like. In some cases, the synthetic peptides comprise one or more or two or more amino acid residues independently selected from the group consisting of Asparagine and Glutamine. In some cases, the synthetic peptides comprise one or more or two or more amino acid residues independently selected from the group consisting of Serine and Threonine. In some cases, the synthetic peptides can be devoid of amino acid residues having chargeable sidechains such as, for example, Lysine, Arginine, Aspartic acid, Glutamic acid, and the like.

The synthetic peptides may include selected hydrophobic amino acid residues including Valine, Isoleucine, Leucine, Alanine, Glycine, and the like.

The synthetic peptides can be devoid of easily oxidizable & unstable amino acid residues such as methionine, cysteine, etc.

In some cases, the synthetic peptides can be devoid of synthetically difficult amino acid residues which are prone to racemization such as, for example, cysteine and histidine.

The synthetic peptides are designed to furnish acceptable signal sensitivity both in positive and negative polarity.

The synthetic peptides are designed to minimize peptide-peptide interactions. This feature can help calibrator composition stability and avoid aggregation. In some cases, specific synthetic peptides are designed to give rise to multiply-charged ions to be used for EAD (Electron Activated Dissociation) calibrations. In some cases, the synthetic peptides are designed to avoid unwanted adduct formation.

A systematic strategy was used to design the synthetic peptides with all the above characteristics incorporated. The desired peptides can be designed by initially constructing an all-glycine peptide framework (Fig. 1), for example, in a chemical drawing program such as ChemDraw™, and then adding suitable amino acid side

chains with desired molecular masses, solubility enhancing functional groups, and one or two UV chromophores etc. A separate side chain chart with respective molecular weights was created (Fig. 2) to assist the exercise of adding, shuffling and exchanging amino acid residues until the desired peptide which satisfies the above criteria has been designed. The peptides were then synthesized and subsequently tested for the solubility in suitable solvent systems, mass spec behavior, UV absorption, etc. to make sure that these compounds pass all the specifications required for being used as efficient calibrators. Fig. 3 depicts a representative list of synthetic peptides designed, synthesized and evaluated for using as mass calibrants.

The synthetic peptides provided herein have substantial advantages over existing calibrants and can be easily washed out of the mass spectrometer system. The disclosure provides synthetic peptides that exhibit predictable MS/MS pattern of b ions and y ions which can be effectively used for both MS2 and MS3 hardware calibration of mass spectrometers. Peptides can be synthesized with consistent purity from various vendors and the variability of batch-to-batch purity is minimum comparing with polymer based calibrants like polypropylene glycol polymers (PPGs) and cyclodextrins. The heavy isotopic versions of the synthetic peptides can be sourced from various vendors making the mass spectrometry-based quality controls (QCs) of the final formulation and the stock solutions reliable and consistent.

Definitions

The phrase "a" or "an" used in conjunction with the applicants' teachings with reference to various elements encompasses "one or more" or "at least one" unless the context clearly indicates otherwise.

The term “about” refers to +/- 10% of the unit value provided.

The term “aqueous organic solvent system” refers to a mixture comprising water and a water-miscible organic solvent. In some cases, the mixture of water and water-miscible organic solvent is in a range of 99:1 to 1:99, 90:10 to 10:90, 80:20 to 20:80, 70:30 to 30:70, 60:40 to 40:60, 55:45 to 45:55, or about 50:50 v:v ratio. In some

cases, the water-miscible organic solvent may be selected from the group consisting of acetonitrile, methanol, ethanol, isopropanol, n-propanol, and the like. In some cases, the aqueous organic solvent system comprises a mixture of water and acetonitrile and optionally one or more modifiers.

The term "aromatic amino acid" refers to an amino acid that contains an aromatic ring. Among the naturally occurring amino acids, aromatic amino acids may include phenylalanine, tryptophan, and tyrosine. In some cases, the aromatic amino acid is selected from the group consisting of phenylalanine and tryptophan.

The term "naturally occurring peptide, protein, or fragment thereof" refers to a peptide, protein, or fragment thereof that occurs in nature. The naturally occurring peptide, protein or fragment thereof may be a recombinant peptide, protein, or fragment thereof. The naturally occurring peptide, protein or fragment thereof may be an isolated peptide, protein, or fragment thereof. The naturally occurring peptide, protein or fragment thereof may be an isolated recombinant peptide, protein, or fragment thereof. In some cases, the naturally occurring peptide, protein or fragment thereof is selected from the group consisting of iPD1 peptide, des-Arg^1-bradykinin, angiotensin I, angiotensin II, substance P, bombesin, Glu^1-fibrinopeptide B, ACTH (1-17 clip), ACTH (18-39 clip), ACTH (7-38 clip), somatostatin, neurotensin, renin, and the like.

The term "natural amino acid" or "naturally occurring amino acid" refers to amino acids that are encoded or proteinogenic amino acids including 20 in the standard genetic code and an additional 2: selenocystiene (Sec, U) and pyrrolysine (Pyl, O) that can be incorporated by special translation mechanisms. Naturally-occurring amino acid residues include L-alanine (Ala, A), L-arginine (Arg, R), L-asparagine (Asn, N), L-aspartic acid (Asp, D), L-cysteine (Cys, C), L-glutamic acid (Glu, E), L-glutamine (Gln, Q), glycine (Gly, G), L-histidine (His, H), L-isoleucine (Ile, I), L-leucine (Leu, L), L-lysine (Lys, K), L-methionine (Met, M), L-phenylalanine (Phe, F), L-proline (Pro, P), L-serine (Ser, S), L-threonine (Thr, T), L-tryptophan (Trp, W), L-tyrosine (Tyr, W), and L-valine (Val, V). Unless otherwise specified, an amino acid or amino acid residue is in the L-configuration. In some cases, the synthetic peptides may comprise naturally occurring amino acid residues selected from the group consisting of

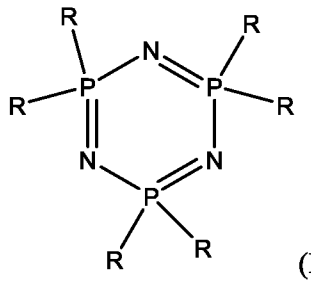
alanine, asparagine, glutamine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, and valine.

The term "unnatural amino acid" refers to non-coded or non-proteinogenic amino acids that are distinct from the 22 proteinogenic amino acids (21 in eukaryotes) including 20 in the standard genetic code and additional 2 (selenocystiene and pyrrolysine) that can be incorporated by special translation mechanisms which are naturally encoded in the genome of organisms for the assembly of proteins. The unnatural amino acids may be chemically synthesized or may occur in nature. The unnatural amino acid may be any compound comprising an amino substituent and a carboxylic acid substituent. The unnatural amino acid may be a chemically modified natural amino acid, e.g., N-alkylated, O-alkylated, or esterified natural amino acid. The unnatural amino acid may be a non-alpha amino acid such as, for example, beta-alanine, gamma-aminobutyric acid, delta-aminolevulinic acid, 4-aminobenzoic acid, and the like. The unnatural amino acid may also be selected from homoalanine, norvaline, or norleucine, The unnatural amino acids may include amino acids having an alpha-carbon in the D configuration (D-amino acids). In some cases, the synthetic peptides may comprise unnatural amino acid residues selected from the group consisting of D-alanine, D-asparagine, D-glutamine, D-isoleucine, D-leucine, D-phenylalanine, D-serine, D-threonine, D-tryptophan, and D-valine.

The term "organic calibrant compounds" refers to a non-peptide organic compound useful for calibrating a mass spectrometer. Organic calibrant compound may be selected from any suitable organic compound. The organic calibrant compound may have a molecular weight in a range between 58 g/mol and 2200 g/mol. In some cases, the organic calibrant compound is selected from the group consisting of imidazole, betaine, pentafluoropropionic acid, reserpine, sodium dodecyl sulfate, tris-fluoromethyl benzoic acid, triacetyl-beta-cyclodextrin, taurocholic acid, and discrete length polyethylene glycol derivatives having a single ionizable functional group that can be ionized by an ion source. These types of discrete length polyethylene glycol derivatives comprise a polyethylene glycol compound having a defined chain length and molecular weight, unlike conventional polyethylene glycol compounds that

comprise a number of compounds having a range of molecular weights due to the nature of polymerization of ethylene oxides. For example, a discrete length polyethylene glycol labelled as PEG5, as an example, is a polyethylene glycol until having five polyethylene oxide units. In some cases, the organic calibrant compound can be a derivatized discrete length polyethylene glycol such as a discrete length polyethylene glycol amine, discrete length polyethylene glycol carboxylic acid, discrete length polyethylene glycol sulfonic acid derivatives, or discrete length polyethylene glycol phenolic derivatives. Suitable discrete length polyethylene glycol derivatives are described in WO2023/021381, which is incorporated herein by reference in its entirety. In some cases, the discrete length polyethylene glycol is a discrete length polyethylene glycol amine. In some embodiments, the method comprises a calibrant composition comprises a discrete length polyethylene glycol amine compound, a discrete length polyethylene glycol -carboxylic acid compound, a discrete length polyethylene glycol - sulfonic acid compound, and a discrete length polyethylene glycol -phenolic compound. In embodiments the method comprises a calibrant composition that comprises a plurality (e.g., 2, 3, 4, or 5, or more, etc.) of discrete length polyethylene glycol amine compounds, discrete length polyethylene glycol -carboxylic acid compounds, discrete length polyethylene glycol -sulfonic acid compounds, and discrete length polyethylene glycol - phenolic compounds.

The term "phosphazene" or "phosphazine" refers to classes of phosphorus compounds comprising phosphorus(V) with a double bond between P and N. The phosphazene may be organic or inorganic. Phosphazenes such as Ultramark 1621® (Thermo Scientific Chemicals) have been described as reference compounds in positive and negative ion fast-atom bombardment high-resolution mass spectrometry (Jiang and Moini, J Am Mass Spectrom 1992, 3, 842-846). The phosphazene may be an optionally halogenated organophosphorus compound. The phosphazene may be a hexakis phosphazene. In some cases, the phosphazene may have a chemical structure according to Formula (I):



(I), wherein R=$CH_2(CF_2CF_2)_nH$, n=1, 2, or 3.

In some cases, the phosphazene may be selected from the group consisting of hexakis(2,2,3,3-tetrafluoropropoxy)phosphazene, hexakis(1H,1H,3H-perfluoropropoxy)phosphazene (phosphazene-921), hexakis(1H, 1H, 4H-butyloxy)phosphazene, hexakis(1H, 1H, 6H-decafluorohexyloxy)phosphazene, hexakis(1H, 1H, 5H-octafluoropentoxy)phosphazene (phosphazene-1521), Hexakis(1H, 1H, 7H-dodecafluoroheptoxy)phosphazine (phosphazene-2121), hexakis(1H, 1H, 8H-tetradecfluorooxtyloxy)phosphazene, hexamethoxyphosphazene, hexakis(1H, 1H, 9H-perfluorononyloxy)phosphazene, and the like. Phosphazene calibrants are commercially available, for example, from Agilent Technologies or Thermo Scientific Chemicals. In some cases, the calibrant composition of the present disclosure does not include a phosphazene compound.

The terms "b ions" and "y ions" refers to certain types of peptide fragments that occur along the peptide backbone after dissociation in the mass spectrometer. The location the fragmentation occurs and the nature of the remaining ion results in various ions a, b, c and x, y, or z ions. The most commonly occurring ions are a, b and y ions. The b ions extend from the amino terminus (N-terminus), the y ions extend from the carboxyl terminus (C-terminus). The "a ions" are often used as a diagnostic for b ions, such that a-b pairs are separated by 28 u, the mass for the carbonyl, C=O.

Solvent systems

In example embodiments in accordance with the disclosure the compositions comprise a solvent to dissolve the calibrant synthetic peptides and can comprise aqueous and/or organic solvents. In some embodiments the solvent can comprise water, organic solvents, or mixtures thereof (e.g., mixtures of miscible organic solvents or

water with miscible organic solvents). In some further embodiments, non-limiting examples of solvents include water, and organic solvents such as acetonitrile, methanol, ethanol, propanol, isopropanol, butanol, dichloromethane, acetone, toluene, benzene, chloroform, dimethylformamide, or aqueous dilutions/combinations thereof. Various pH modifiers such as organic acids (e.g., formic acid, acetic acid, difluoroacetic acid, trifluoroacetic acid, hexafluoroisopropanol), amines (e.g., triethylamine, dimethyl amine), or salts thereof (e.g., ammonium formate, ammonium acetate) may be included. The solvent system may be an aqueous organic solvent system. The aqueous organic solvent system comprises water and a water-miscible organic solvent, for example, in a range of 99:1 to 1:99, 90:10 to 10:90, 80:20 to 20:80, 70:30 to 30:70, 60:40 to 40:60, 55:45 to 45:55, or about 50:50 v:v ratio v/v. In some further embodiments, the solvent can comprise a mixture of water and one or more water-miscible organic solvents such as acetonitrile, methanol, isopropanol, ethanol, and n-propanol, or combinations thereof. In some embodiments, the solvent can comprise an aqueous organic mixture of acetonitrile and water; acetone and water; ethanol and water; methanol and water. The solvent system may comprise a mixture of water and acetonitrile. The solvent system may comprise a mixture of water and acetonitrile and one or more modifiers. The modifier may be a modifier selected from the group consisting of formic acid, acetic acid, ammonium formate, ammonium acetate, triethylamine, dimethyl amine, trifluoroacetic acid, difluoroacetic acid, and hexafluoroisopropanol. pH modifiers may be selected from, for example, formic acid, acetic acid, triethylamine, dimethyl amine, trifluoroacetic acid, difluoroacetic acid, and hexafluoroisopropanol. Acidic pH modifiers may be selected from, for example, formic acid, acetic acid, trifluoroacetic acid, difluoroacetic acid, and hexafluoroisopropanol. The calibrant compositions may comprise one or more acidic modifier compounds such as formic acid, acetic acid, difluoroacetic acid, or hexafluoroisopropanol, and the like. In some cases, the solvent system may include an acidic modifier such as formic acid or acetic acid in about 0.01-0.2 vol%, 0.5-0.15 vol%, or about 0.1 vol%.

In some cases, the solvent system includes a modifier such as ammonium formate or ammonium acetate which may be employed for consistent calibrant adduct formation. The modifier such as ammonium formate or ammonium acetate may be in a

range between about 0.05 and about 0.50 mM, about 0.10 mM and about 0.40 mM, about 0.2 and about 0.3 mM, or about 0.25 mM.

The solvent system may comprise a mixture of water and acetonitrile with modifier formic acid. The solvent system may comprise a mixture of water and acetonitrile with modifiers formic acid and ammonium formate.

Compositions

Calibrant compositions are provided for calibrating a mass spectrometer. The mass spectrometer may be, for example, an electrospray ionization (ESI) mass spectrometer, an atmospheric pressure chemical ionization (APCI) mass spectrometer, fast atom bombardment (FAB) mass spectrometer, or a matrix-assisted laser desorption ionization (MALDI) mass spectrometer.

Calibrant compositions comprising one or more, two or more, three or more, or four or more of the synthetic peptides are provided. Calibrant compositions may include the synthetic peptides, and one or more organic calibrant compounds. Calibrant compositions may include the synthetic peptides, one or more organic calibrant compounds, and one or more naturally occurring peptides, proteins, or fragments thereof. The calibrant components are selected to span a desirable range of molecular masses. In some cases, the calibrant components may be selected to span a range of molecular masses between 100 and 3500 Da, 100 and 2500 Da, or 100 and 2200 Da.

The calibrant compositions may comprise one or more, two or more, three or more, or four or more synthetic peptides each in a concentration of between about 0.1 and about 20 micromolar (uM), about 0.5 and about 10 uM, or about 1 and about 5 uM.

The calibrant compositions may comprise one or more naturally occurring peptides, proteins, or fragments thereof, each in a concentration of between about 0.1 and about 20 micromolar (uM), about 0.5 and about 10 uM, or about 1 and about 5 uM.

The calibrant compositions may comprise one or more, two or more, three or more, or four or more organic calibrant compounds, each in a concentration of between

about 0.1 and about 100 micromolar (uM), about 0.2 and about 50 micromolar (uM); about 0.3 and about 30 uM, or about 1 and about 10 uM.

The calibrant compositions may comprise one or more, two or more, three or more, or four or more mobile phase modifier compounds such as ammonium formate or ammonium acetate, each in a concentration of between about 0.01 and about 1.00 mM; about 0.05 and about 0.50 mM; about 0.1 and about 0.40 mM, about 0.2 and about 0.3 mM, or about 0.25 mM.

The calibrant compositions may comprise one or more, two or more, three or more, or four or more acidic mobile phase modifier compounds such as formic acid, acetic acid, difluoroacetic acid, hexafluoroisopropanol, each in a range of between about 0.01% and about 0.5 vol%; about 0. 03% and 0. 5 vol%; about 0.05% and about 0.2%; or about 0.1 vol%.

The calibrant compositions may comprise a solvent system suitable for solvation of each of the calibrant components and suitable for mass spectrometry analysis of the calibrant components. The solvent system may be an aqueous solvent system, an organic solvent system, or an aqueous organic solvent system. In some cases, the aqueous organic solvent system comprises a mixture of water and water-miscible organic solvent is in a range of 99:1 to 1:99, 90:10 to 10:90, 80:20 to 20:80, 70:30 to 30:70, 60:40 to 40:60, 55:45 to 45:55, or about 50:50 v:v ratio. In some cases, the water-miscible organic solvent may be selected from the group consisting of acetonitrile, methanol, ethanol, isopropanol, n-propanol, and the like. In some cases, the aqueous organic solvent system comprises a mixture of water and acetonitrile and optionally one or more mobile phase modifiers. The one or more mobile phase modifiers may be volatile modifiers selected from the group consisting of formic acid, acetic acid, trifluoroacetic acid, difluoroacetic acid, triethylamine, dimethylamine, ammonium formate, ammonium acetate, and the like. The one or more mobile phase modifiers may be modifiers selected from the group consisting of formic acid, acetic acid, ammonium formate, and ammonium acetate. In some cases, the solvent system comprises water and acetonitrile. In some cases, the solvent system comprises water,

acetonitrile and formic acid. In some cases, the solvent system comprises water, acetonitrile, formic acid, and ammonium formate.

In some cases, calibrant compositions may include a plurality of synthetic peptides; one or more organic calibrant compounds; and a solvent system. In some cases, calibrant compositions may include a plurality of synthetic peptides; one or more naturally occurring peptides, proteins, or fragment thereof; and a solvent system. In some cases, calibrant compositions may include a plurality of synthetic peptides; one or more organic calibrant compounds; one or more naturally occurring peptides, proteins, or fragment thereof; and a solvent system. The organic calibrant compounds may include one or more of discrete length polyethylene glycol amines.

Stability of the calibrant compositions may be determined, for example, for each of the synthetic peptides by mass spectrometry (MS), liquid chromatography-mass spectrometry (LC-MS), LC-MS/MS, or 100% ±20% starting peak area by high performance liquid chromatography (HPLC).

The calibrant compositions disclosed herein have good stability (shelf- and shipping-stable) and can remain stable for weeks at ambient temperatures (e.g., 8 weeks at 25 °C with excursions to 45 °C). The calibrant compositions may be premixed in the solvent at ready-to-use concentrations for performing mass spec calibration. the calibrant compositions may be stored at reduced temperature (e.g., 4 °C or other refrigerated temperatures) to help prolong composition stability. Under refrigerated storage conditions, the calibrant compositions are stable for a period of at least 3 months when stored at 4 °C. In some cases, the calibrant composition may be stable for at least 6 months, or at least 12 months when stored at 4 °C.

Methods

The disclosure provides methods for calibrating, optimizing, and tuning parameters of a mass spectrometer to ensure and/or increase the accuracy of mass spectroscopy analysis, including tandem mass spectroscopy analysis. The mass spectrometer for calibration may be an electrospray ionization (ESI) mass spectrometer,

an atmospheric pressure chemical ionization (APCI) mass spectrometer, fast atom bombardment (FAB) mass spectrometer, or a matrix-assisted laser desorption ionization (MALDI) mass spectrometer.

In some embodiments the method comprises obtaining at least one mass spectrum of a plurality of synthetic peptides and/or a calibration composition as disclosed herein. In some embodiments the method comprises obtaining at least one mass spectrum of a calibrant composition as mass standards, for example an internal or external standard. In some embodiments, the method comprises a calibrant composition as an internal standard. In some embodiments, the method comprises a calibrant composition as an external standard.

In performing a method in accordance with the aspects and embodiments of the disclosure, the differences between the calculated theoretical mass peaks for the known synthetic peptides and the corresponding mass peaks obtained can be determined and the mass spectrometer can be adjusted based on the differences between the expected and actual mass peaks. In various embodiments, the method comprises acquiring a mass spectrum of a calibrant composition on a mass spectrometer that is operating in either positive or negative ionization mode. In some embodiments, the methods comprise calibrating an APCI or an electrospray mass spectrometer.

In some embodiments, the methods can comprise one or more calibration compositions having different concentrations, or various dilutions of a calibration composition. In such embodiments, the method can comprise calibrating mass spec based on the acquired signal intensity and the expected signal intensity based on synthetic peptide concentrations. In related embodiments the calibrant compositions may be diluted when used in methods for calibrating mass spectrometers capable of detecting low level analytes (e.g., high-sensitive instrumentation such as the Triple Quad 7500 System from Sciex). In some embodiments, the methods can comprise an autotune program or other similar type of automated MS acquisition application that may be included with mass spec operational software. In embodiments, the methods and compositions can be used to calibrate one or more quadrupoles and may be used in any variety of MS scanning modes including, for example, MS, MS/MS, product ion,

precursor ion, neutral loss or gain, MRM, EMS, EPI, ER, MS 3, or MRM3 scanning modes. For example, in such embodiments, the methods and compositions allow for calibration of single ion monitoring scans, full scans, mass-filter/fragmentation scans, and multiple reaction monitoring scans, among others.

The methods can comprise a calibrant composition comprising a plurality of known compounds comprising a mixture of synthetic peptides that can calibrate a variety of mass spec instruments that use various ion sources and that can operate in negative or positive ionization mode. In some embodiments the calibrant composition comprises at least one synthetic peptide and one or more of organic calibrant compounds and one or more naturally occurring peptide, protein, or fragment thereof as the calibrant compounds. In some embodiments the calibrant composition does not include a phosphazene compound.

In various embodiments, the methods can enable the calibration of a mass spectrometer across a broad range of masses, or narrower mass ranges within a broader range. In various embodiments, the methods enable accurate and consistent calibration across a mass range of about 100 to about 3500 Da, or about 100 to about 3000 Da, or about 100 to about 2500 Da, or about 100 to about 2400 Da, or about 100 to about 2300 Da, or about 100 to about 2200 Da, or about 100 to 2100 Da, or about 100 to 2000 Da, or about 50 to about 1500 Da, or within a narrower mass range that falls within any of the above ranges (e.g., about 500-1000 Da, 250-1500 Da, 750-2500 Da, etc.). In some embodiments, the method comprises a calibrant composition comprising a plurality of synthetic peptides, organic calibrant compounds, and one or more naturally occurring peptide, protein, or fragment thereof that create a molecular weight "ladder" that comprise molecular weights that span regular intervals within a defined mass range.

Kits

The disclosure provides a kit comprising the compositions disclosed herein. In some embodiments, the kit may comprise a kit of parts that comprises, for example, a plurality of synthetic peptides in single use container for calibrating a mass spectrometer. In embodiments, the kit can comprise a predetermined concentration of

one or a plurality of synthetic peptides or a calibrant composition comprising a mixture of various synthetic peptides in accordance with the disclosure, and a solvent system. In some cases, the calibrant composition is provided in a ready-to-use concentration or in a concentrated form for dilution. In some cases, the kit comprises a separate container with a solvent system.

In various embodiments, the kits may be used in methods for calibrating a mass spectrometer such as, for example, an electrospray ionization (ESI) mass spectrometer, an atmospheric pressure chemical ionization (APCI) mass spectrometer, fast atom bombardment (FAB) mass spectrometer, or a matrix-assisted laser desorption ionization (MALDI) mass spectrometer. In such embodiments, the kits provide for such calibration in either the positive ionization mode or the negative ionization mode. In the aspects and embodiments relating to kits, the disclosure provides for any combination of one or more synthetic peptides described herein. In some embodiments, the kit can comprise a solvent system that is effective to dissolve plurality of synthetic peptides and/or the calibrant composition. In such embodiments, the solvent system can comprise any of suitable solvent or mixtures thereof in accordance with the disclosure and example embodiments provided herein.

In various embodiments, the kit comprises a plurality of synthetic peptides and/or a calibrant composition comprising a mixture of synthetic peptides that provides for calibration of a mass spectrometer across a broad range of masses. In some embodiments, the kit can comprise a plurality of synthetic peptides and/or a calibrant composition that can enable calibration across a range of approximately 100 to 3500 Da, or in some embodiments from about 100 to about 2200 Da, in either positive ionization mode or negative ionization mode.

EXAMPLES

Example 1. Design of Synthetic Peptide Calibrants

Earlier generation calibrant formulations include undesirable components cesium iodide, amino- discrete length polyethylene glycol -acids, and phosphazenes.

Cesium iodide suffered from lack of UV chromophore, linearity issues, and undesirable adduct formation. Amino- discrete length polyethylene glycol acids including amino-PEG4-acid, amino-PEG6-acid, and amino-PEG8-acid suffered from field stability issues. Phosphazenes, including phosphazene-921, phosphazene-1521, and phosphazene-2121, suffered from no UV chromophore and carryover concerns.

A series of synthetic peptides was designed to replace undesirable calibrant components of suitable mass, solubility, stability, and comprising a UV chromophore to impart an orthogonal method of quantification.

The desired peptides were designed by initially constructing an all-glycine peptide framework of an appropriate length (Fig. 1), for example, in chemical drawing program ChemDraw™ and then adding suitable amino acid side chains with desired molecular masses, solubility enhancing functional groups, and one or two UV chromophores. A side chain chart with respective molecular weights was created (Fig. 2) to assist the exercise of adding, shuffling and exchanging amino acid residues until the desired peptide which satisfies the above criteria has been designed.

The amino acid side chains were selected from those of alanine, asparagine, glutamine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, and valine. Use of chargeable side chains of lysine, arginine, aspartic acid, or glutamic acid was avoided to minimize multiply charged ions and enhance stability. Use of side chains of easily oxidizable & unstable amino acid residues such as methionine, cysteine, or those prone to racemization such as, for example, cysteine and histidine were avoided.

The synthetic peptides were synthesized and tested for acceptable solubility in suitable solvent systems, mass spec behavior, UV absorption, stability to make sure that the candidate synthetic peptides passed each of the specifications required for being used as efficient calibrators. Fig. 3 depicts a representative list of synthetic peptides designed, synthesized and evaluated for using as mass calibrants including 380-peptide (SEQ ID NO: 1); 477-peptide (SEQ ID NO: 2); 618-peptide (SEQ ID NO: 3); 823-peptide (SEQ ID NO: 4); 833-peptide (SEQ ID NO: 5); 919-peptide (SEQ ID NO: 6);

923-peptide (SEQ ID NO: 7); 1520-peptide (SEQ ID NO: 8); 1522-peptide (SEQ ID NO: 9); 1524-peptide (SEQ ID NO: 10); and 2219-peptide (SEQ ID NO: 11).

Various calibrant compounds in a range of 100 to 2200 Da were selected for development of calibrant compositions including one or more of the synthetic peptides.

Example 2. Calibrant Composition 1

Calibrant composition 1 was prepared including seven synthetic peptides selected from table 1 including 380-peptide (SEQ ID NO: 1); 477-peptide (SEQ ID NO: 2); 618-peptide (SEQ ID NO: 3); 833-peptide (SEQ ID NO: 5); 919-peptide (SEQ ID NO: 6); 1520-peptide (SEQ ID NO: 8); and 1522-peptide (SEQ ID NO: 9) and subjected to mass spectrometry analysis in positive ion (+TOF-MS) and negative ion (-TOF-MS) modes.

FIG. 4A shows positive ion mode mass spectrum for calibrant composition 1 in different dilutions 1X (1uM, +TOF-MS, 100-2000 Da, upper panel), 3X (middle panel), and 5X (lower panel). Each vertical bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar represents the relative abundance of the ion. The most intense ion is assigned an abundance of 100 and can be referred to as the base peak.

FIG. 4B shows negative ion mode mass spectrometry data for the selected panel of seven synthetic peptides from table 1 in different dilutions 1X (1uM, -TOF-MS, 100-2000 Da, upper panel), 3X (middle panel), and 5X (lower panel).

Example 3. Calibrant Composition 2

Calibrant composition 2 was prepared according to table 2 (FIG. 5). Calibrant composition 2 includes three synthetic peptides according to the disclosure including 477-peptide (SEQ ID NO: 2) which was used to replace unstable amino-PEG8-acid, 923-peptide (SEQ ID NO: 7) which was used to replace phosphazene-921, and 1524-peptide (SEQ ID NO: 10) which was used to replace phosphazene-2121. Additional components of calibrant composition 2 include imidazole, betaine,

pentafluoropropionic acid, m-PEG5-amine, sodium dodecyl sulfate, amino-PEG8-alcohol, reserpine, iPD1 peptide and triacetyl-beta-cyclodextrin, as shown in Table 2.

FIG. 6A shows positive ion mode mass spectrum for calibrant composition 2 (+TOF-MS, 100-2300 Da) including three synthetic peptides 477-peptide (SEQ ID NO: 2), 923-peptide (SEQ ID NO: 7), and 1524-peptide (SEQ ID NO: 10). The inset shows spectrum from formulation component triacetyl-beta-cyclodextrin (+TOF-MS, 2034-2044Da).

FIG. 6B shows negative ion mode mass spectrum for calibrant composition 2 (-TOF-MS, 100-2300 Da) including three synthetic peptides 477-peptide (SEQ ID NO: 2), 923-peptide (SEQ ID NO: 7), and 1524-peptide (SEQ ID NO: 10). The inset shows spectrum from formulation component triacetyl-beta-cyclodextrin (-TOF-MS, 2060-2066Da).

Unlike phosphazene compounds, the synthetic peptides in calibrant composition 2 did not suffer from carryover in the mass spectrometer.

Example 4. Calibrant Composition 3

Calibrant composition 3 was prepared according to table 3 (FIG. 7). Three different formulae A, B, and C were employed using modified concentrations of components. Calibrant composition 3 includes four synthetic peptides according to the disclosure including 477-peptide (SEQ ID NO: 2), 618-peptide (SEQ ID NO: 3); 923-peptide (SEQ ID NO: 7); and 1524-peptide (SEQ ID NO: 10). Additional components of calibrant composition 3 include imidazole, betaine, pentafluoropropionic acid, m-PEG5-amine, sodium dodecyl sulfate, amino-PEG8-alcohol, iPD1 peptide and triacetyl-beta-cyclodextrin, as shown in Table 2. The solvent system includes acetonitrile/water (50:50 v/v), 0.1% by vol formic acid, and 0.25 mM ammonium formate to promote consistent cyclodextrin-ammonium adduct formation.

FIG. 8 shows a negative ion mass spectrum (-TOF-MS) in a range of 100-2200 from 0.511 to 0.921 min of calibrant composition 3, formula B. The inset table shows m/z and sum intensities for the calibrant composition.

The calibrant composition 3, formula B was stable for at least 8 weeks at 25 °C with excursions to 45 °C in ready-to-use concentration, and at least 3 months when stored at 4 °C.

The above specification, examples and data provide a complete description of the manufacture and use of the composition of the invention. Since many embodiments of the invention can be made without departing from the spirit and scope of the invention, the invention resides in the claims hereinafter appended.

WHAT IS CLAIMED IS:

1. A composition for calibration of a mass spectrometer in either positive or negative mode, comprising a plurality of synthetic peptides each comprising: a molecular weight in a range of 220 g/mol to 2200 g/mol and at least one amino acid residue comprising an aromatic side chain.

2. The composition of claim 1, wherein each of the synthetic peptides is soluble in an aqueous buffer in at least 1 mg/mL, or at least 2 mg/mL at 4 deg C.

3. The composition of claim 1 or claim 2, wherein the synthetic peptides each comprise from 3 to 23 naturally occurring amino acid residues or unnatural amino acid residues.

4. The composition of claim 3, wherein the naturally occurring amino acid residues are selected from the group consisting of alanine, asparagine, glutamine, glycine, isoleucine, leucine,-phenylalanine, serine, threonine, tryptophan, and valine.

5. The composition of claim 4, wherein the synthetic peptides each comprise at least one amino acid residue selected from the group consisting of serine and threonine.

6. The composition of claim 4, wherein the synthetic peptides each comprise at least one amino acid residue selected from the group consisting of asparagine and glutamine.

7. The composition of any preceding claim, wherein the amino acid residue comprising an aromatic side chain is selected from the group consisting of phenylalanine and tryptophan.

8. The composition of any preceding claim, wherein the synthetic peptides each do not include an amino acid residue selected from the group consisting of arginine, lysine, aspartic acid, cysteine, glutamic acid, histidine, methionine, proline, and tyrosine.

9. The composition of any preceding claim, wherein each of the synthetic peptides does not include a chargeable amino acid residue.

10. The composition of any preceding claim, wherein the plurality of synthetic peptides comprises two or more, two to six, or three to five of the synthetic peptides.

11. The composition of any preceding claim, further comprising one or more organic calibrant compounds.

12. The composition of claim 11, wherein the one or more organic calibrant compounds comprise a discrete length polyethylene glycol amine terminated with a C_{1-6} alkyl or a hydroxy group.

13. The composition of claim 12, wherein the discrete length polyethylene glycol amine has a molecular weight in a range from 105 g/mol to 692 g/mol.

14. The composition of any preceding claim, further comprising a naturally occurring peptide, protein, or fragment thereof selected from the group consisting of iPD1 peptide, des-Arg^1-bradykinin, angiotensin I, angiotensin II, substance P, bombesin, Glu^1-fibrinopeptide B, ACTH (1-17 clip), ACTH (18-39 clip), ACTH (7-38 clip), somatostatin, neurotensin, and renin.

15. The composition of any preceding claim, wherein the composition does not comprise a phosphazene.

16. The composition of any preceding claim, in a liquid form comprising an aqueous organic solvent system.

17. The composition of claim 16, wherein the liquid form is in a ready-to-use concentration.

18. The composition of claim 16 or claim 17, wherein the aqueous organic solvent system comprises water and a water-miscible organic solvent in a range of 99:1 to 1:99 v/v optionally wherein the water-miscible organic solvent is selected from the group consisting of acetonitrile, methanol, isopropanol, ethanol, and n-propanol, optionally wherein the solvent system comprises a mixture of water and acetonitrile.

19. The composition of any one of claims 16 to 18, wherein the solvent system comprises a mobile phase modifier, optionally wherein the mobile phase modifier is selected from the group consisting of formic acid, ammonium formate, ammonium acetate, triethylamine, trifluoroacetic acid, difluoroacetic acid, and hexafluoroisopropanol.

20. The composition of any one of claims 16 to 19, in a ready-to-use concentration comprising 0.1-10 uM, or 1-4 uM of each of the one or more synthetic peptides.

21. The composition of any of claims 16 to 20, comprising one or more of:

the plurality of the synthetic peptides of claim 1;

one or more of the discrete length polyethylene glycol amines of claim 12 or claim 13;

one or more of the organic calibrant compounds of any one of claims 11-13;

the naturally occurring peptide, protein, or fragment thereof of claim 14; and

the solvent system of any one of claims 16-19.

22. The composition of any one of claims 16 to 21, wherein the composition is stable for at least 3 months at 25 deg C when stored away from light as determined for each of the one or more synthetic peptides by mass spectrometry (MS), liquid chromatography-mass spectrometry (LC-MS), LC-MS/MS, or 100% ±20% starting peak area by high performance liquid chromatography (HPLC).

23. A method for calibrating a mass spectrometer comprising:

obtaining a mass spectrum of the composition for calibration of any one of claims 1 to 22;

determining the differences between the expected mass peaks for each of the plurality of the synthetic peptides and the corresponding actual mass peaks obtained; and

adjusting the mass spectrometer based on the differences between the expected and actual mass peaks.

24. The method of claim 23, wherein the composition for calibration is used in either positive or negative ionization mode.

25. The method of claim 23 or 24, wherein the mass spectrometer is an electrospray ionization (ESI) mass spectrometer, an atmospheric pressure chemical ionization (APCI) mass spectrometer, fast atom bombardment (FAB) mass spectrometer, or a matrix-assisted laser desorption ionization (MALDI) mass spectrometer.

26. The method of any one of claims 23 to 25, wherein the method is performed in MS/MS mode.

27. The method of any one of claims 23 to 26, wherein the composition for calibration calibrates the mass spectrometer across a range of from 100 to 2200 Da or 50 to 1500 Da.

28. A kit comprising:

at least one composition according to any one of claims 1 to 15;

a solvent; and

instructions for use.

29. A method of providing a synthetic peptide calibrant, comprising

selecting a peptide calibrant target mass in a range between 200 Da and 2,200 Da;

selecting a poly-glycine peptide framework having a mass less than the target mass and comprising between 3 and 23 glycine residues;

replacing the alpha proton in one or more, two or more, three or more, or four or more of the glycine residues with an amino acid side chain independently selected from a naturally occurring alpha-amino acid side chain or unnatural amino acid side chain to designate the synthetic peptide calibrant having the target mass; and

synthesizing the designated synthetic peptide calibrant having the target mass.

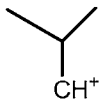
30. The method of claim 29, wherein the naturally occurring alpha-amino acid side chains are independently selected from the group consisting of

Exact Mass: 14.02
Ala

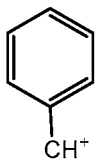
Exact Mass: 30.01
Ser

Exact Mass: 45.03
Thr

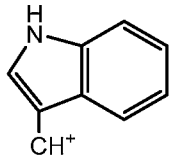
Exact Mass: 42.05
Val



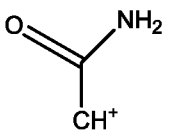
Exact Mass: 56.06
Leu



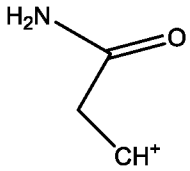
Exact Mass: 90.05
Phe



Exact Mass: 129.06
Trp



Exact Mass: 57.02
Asn



Exact Mass: 71.04
Gln

31. The method of claim 29 or claim 30, wherein the synthetic peptide calibrant comprises at least one amino acid residue comprising an aromatic side chain, selected from the group consisting of phenylalanine and tryptophan.

32. The method of any of claims 29-31, wherein the synthetic peptide calibrant comprises at least one amino acid residue selected from the group consisting of serine and threonine.

33. The method of any of claims 29-32, wherein the synthetic peptide calibrant comprises at least one amino acid residue selected from the group consisting of asparagine and glutamine.

34. The method of any of claims 29-33, wherein the synthetic peptides is soluble in an aqueous buffer in at least 1 mg/mL, or at least 2 mg/mL at 4 deg C.

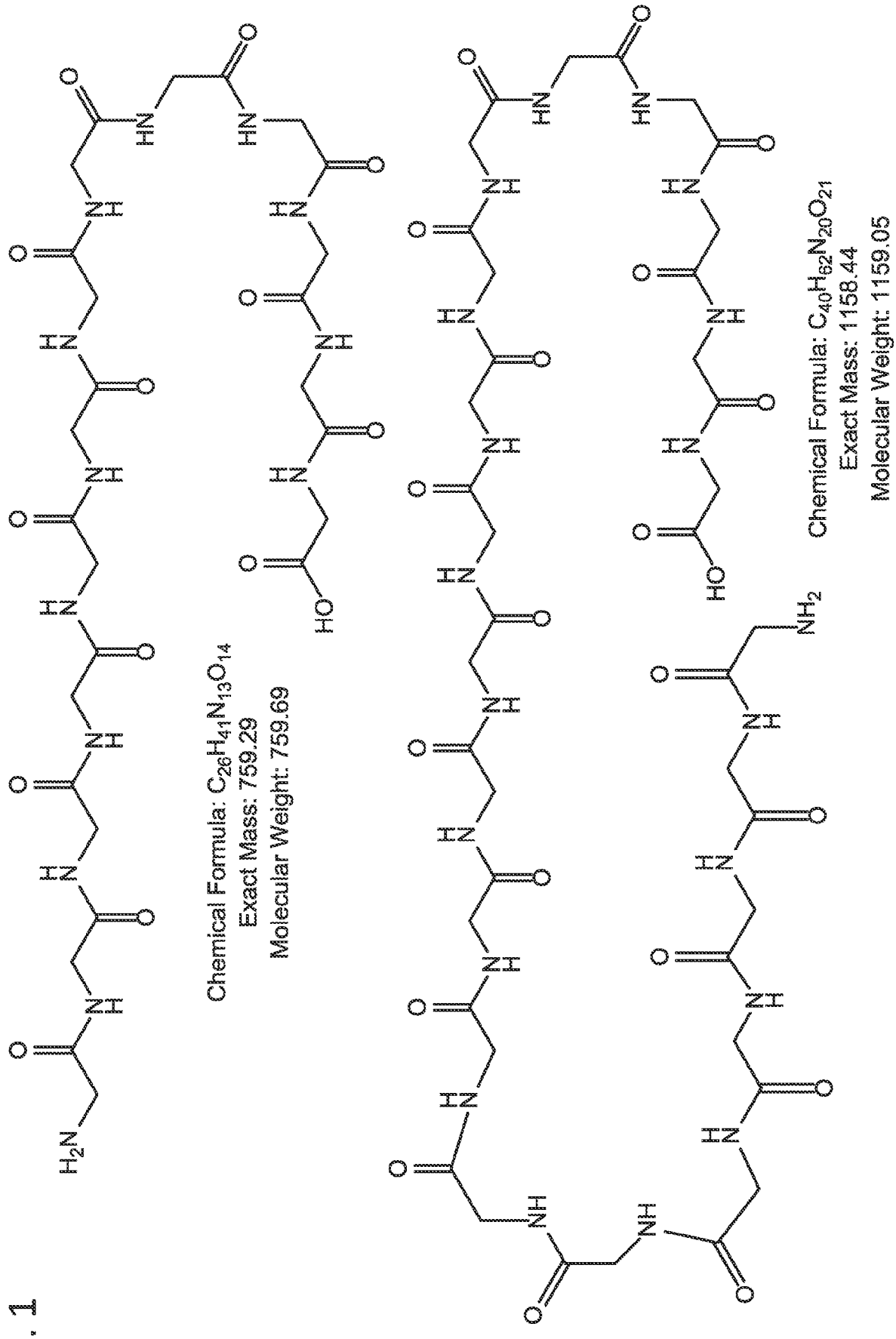
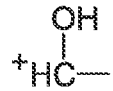

Chemical Formula: $C_{26}H_{41}N_{13}O_{14}$
Exact Mass: 759.29
Molecular Weight: 759.69
Chemical Formula: $C_{40}H_{62}N_{20}O_{21}$
Exact Mass: 1158.44
Molecular Weight: 1159.05

FIG. 1

FIG. 2


Exact Mass: 14.02
Ala

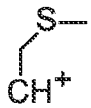

Exact Mass: 30.01
Ser

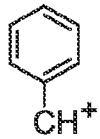

Exact Mass: 45.03
Thr

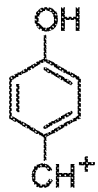

Exact Mass: 60.00
Cys

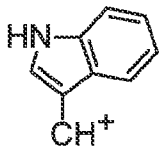

Exact Mass: 42.05
Val

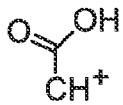

Exact Mass: 56.06
Leu

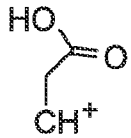

Exact Mass: 74.02
Met

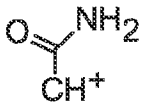

Exact Mass: 90.05
Phe

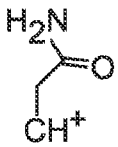

Exact Mass: 106.04
Tyr

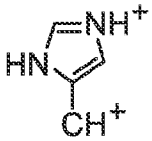

Exact Mass: 129.06
Trp

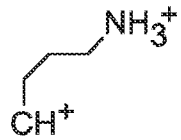

Exact Mass: 58.00
Asp


Exact Mass: 72.02
Glu


Exact Mass: 57.02
Asn


Exact Mass: 71.04
Gln


Exact Mass: 81.04
His


Exact Mass: 72.08
Lys

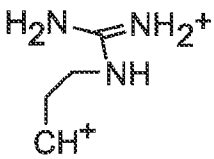

Exact Mass: 100.09
Arg

FIG. 3

Table 1. Synthetic Peptides

Compound				Mass Spec m/z	
ID	Name	SEQ ID NO:	Mol. Wt.	Positive	Negative
380-Peptide	2HN-NFT-OH	1	380.4	381.4	379.4
477-Peptide	H2N-GQWS-OH	2	476.5	477.1	475.1
618-Peptide	2HN-AQGAWS-OH	3	618.6	619.6	617.6
823-Peptide	H2N-ATQASLFS-OH	4	823.9	824.9	823.9
833-Peptide	2HN-ALSGTLWS-OH	5	833.9	834.9	833.9
919-Peptide	2HN-AGQSNWSNG-OH	6	919.9	920.9	918.9
923-Peptide	2HN-AGQSNFSNV-OH	7	923	924	922
1520-Peptide	H2N-GQQGWGSTLQGFVVG-OH	8	1520.7	1521.7 & 760.7	1520.7
1522-Peptide	2HN-AGQSNFSNGKWNGGV-OH	9	1522.6	1524 & 762	1521.6
1524-Peptide	H2N-GQGQFGSLQGFSVGVG-OH	10	1524.7	1525.7475	1522.7353
2219-Peptide	2HN-GQGQFGSLQGFSVGVGFQASGFG-OH	11	2219.4	2220.4 & 1110.4	2218

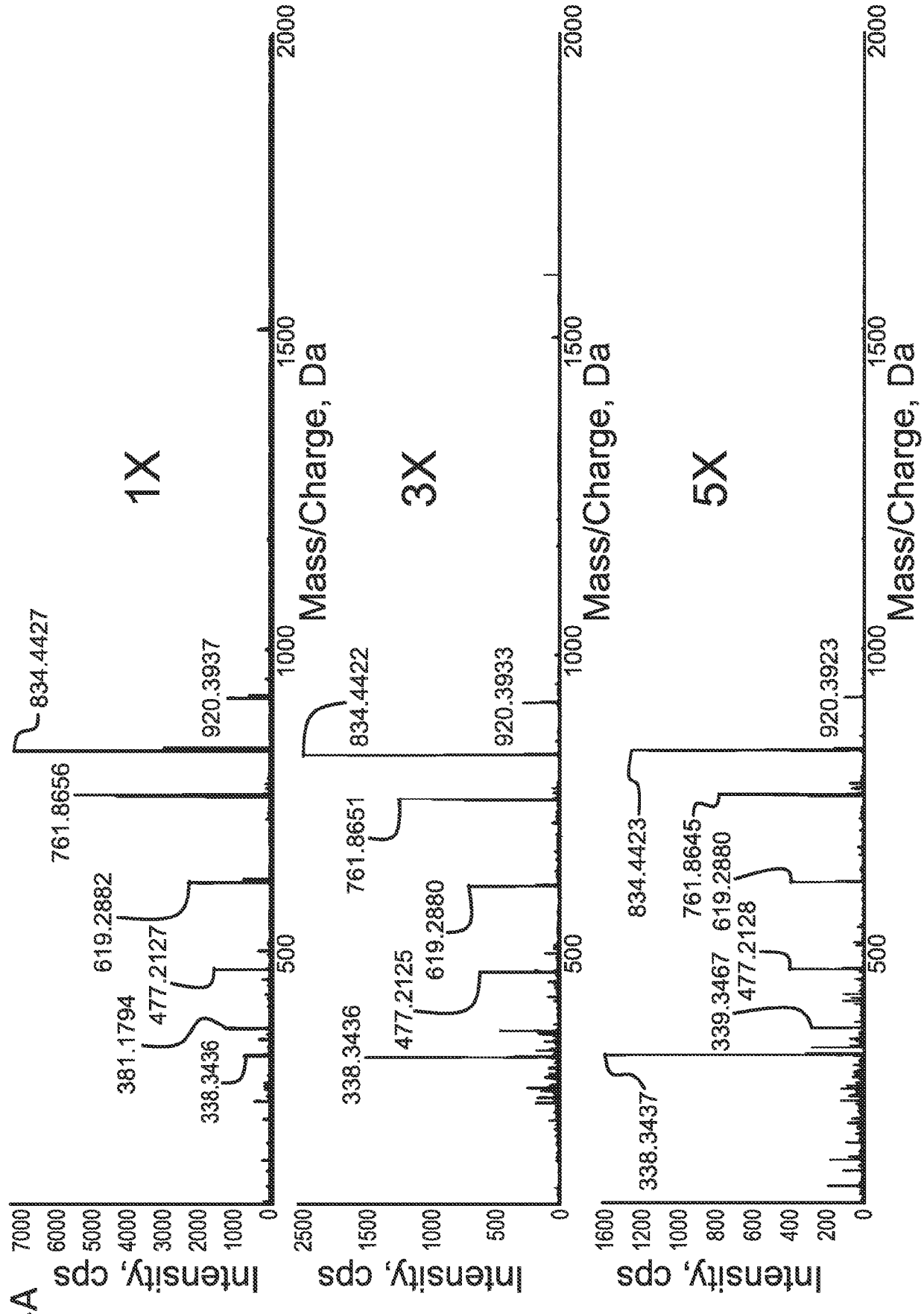

1X
3X
5X
Intensity, cps
Mass/Charge, Da
834.4427
761.8656
619.2882
477.2127
381.1794
338.3436
920.3937
834.4422
761.8651
619.2880
477.2125
338.3436
920.3933
834.4423
761.8645
619.2880
477.2128
339.3467
338.3437
920.3923

FIG. 4A

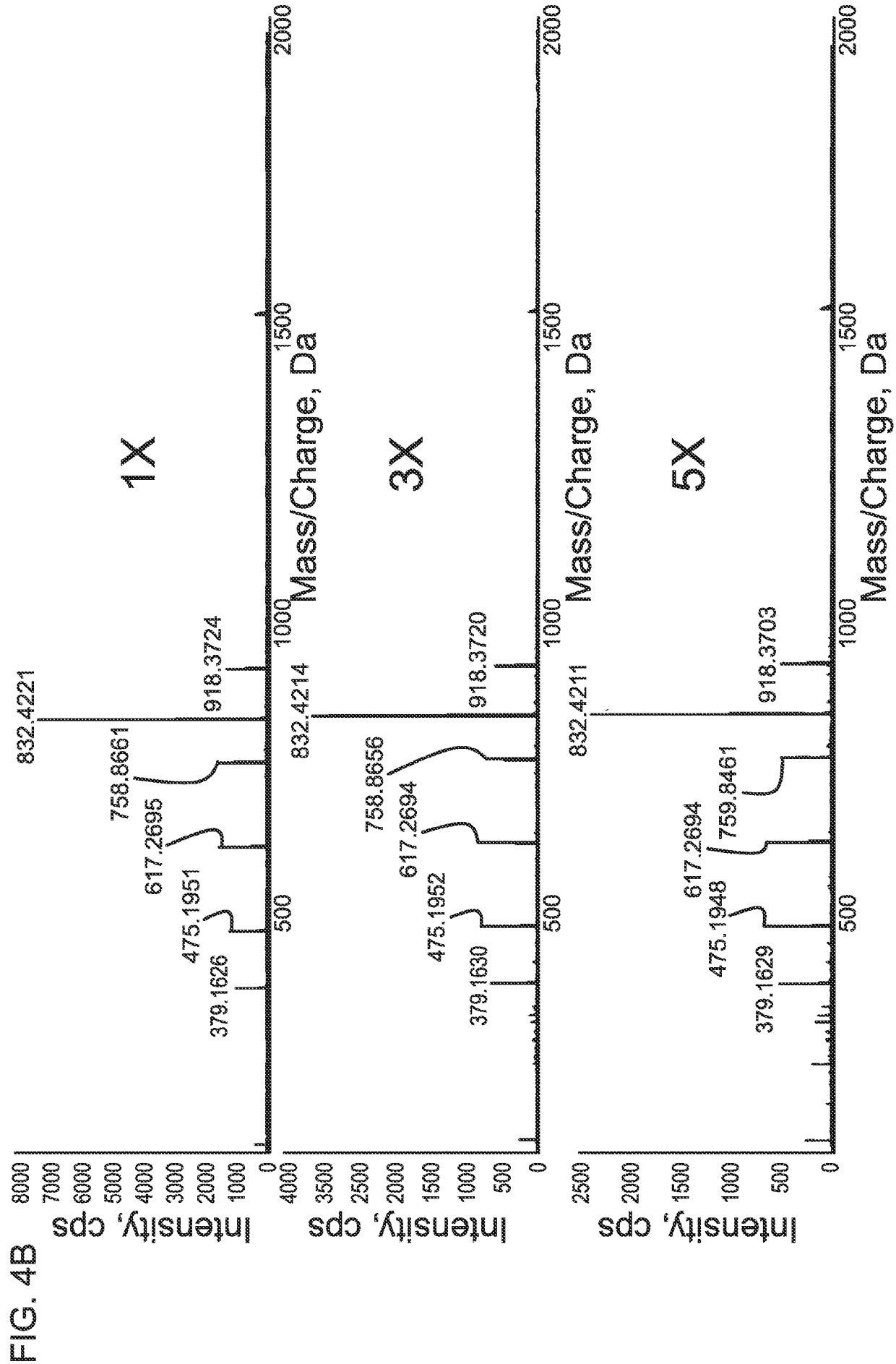

1X
3X
5X
Intensity, cps
Mass/Charge, Da
832.4221
758.8661
617.2695
475.1951
379.1626
918.3724
832.4214
758.8656
617.2694
475.1952
379.1630
918.3720
832.4211
617.2694
759.8461
475.1948
379.1629
918.3703

FIG. 4B

FIG. 5

Table 2. Calibrant Composition 2

Component		Target m/z		Previous Generation Components	
Name	Molecular Mass	Positive	Negative	Aims to Replace	Observed issues
Imidazole	68.1	N/A	N/A	N/A	
Betaine	117.1	118.086804	N.D.	Cesium[iodide]	
Pentafluoropropionic Acid	164	N.D.	162.981845 118.992016	[Cesium] iodide	No chromophore; linearity issues; undesirable adduct formation
m-PEG5-Amine	251.3	252. 181098	N.D.	amino-PEG4-acid	field stability issues reported
Sodium Dodecyl Sulfate	288.4	N. D.	265.147355	N/A	retained component
Amino-PEG8-Alcohol	369.5	370.2440923	N.D.	amino-PEG6-acid	field stability issues reported
H2N-GQWS-OH (SEQ ID NO: 2)	476.5	477.2097724	475. 194122	amino-PEG8-acid	field stability issues reported
Reserpine	608.7	609.2812061	N.D.	N/A	retained component
iPD1 Peptide	829	829.5398805 415.2738528	827.52423	N/A	retained component
H2N-AGQSNFSNV-OH (SEQ ID NO: 7)	922.9	923.4222845 462.2150548	921.406634	Phosphazene-921	No chromophore; carryover concerns
H2N-GQGQFGSLQGFSVGVG-OH (SEQ ID NO: 10)	1524.6	1524.744681 762.8762532	1522. 729031	Phosphazene-1521	No chromophore; carryover concerns
Triacetyl-beta-cyclodextrin	2017.8	2034.625997 1017.816911	2061.589277	Phosphazene-2121	No chromophore; carryover concerns

FIG. 6A

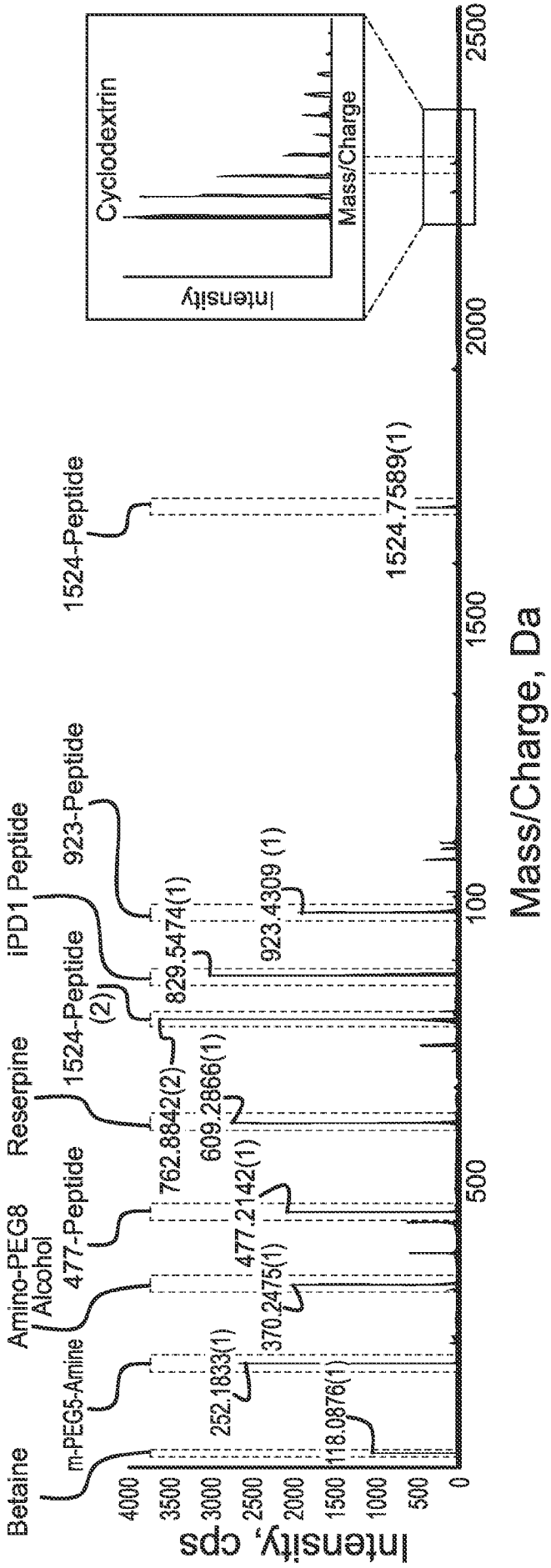

Intensity, cps
Mass/Charge, Da
Betaine
m-PEG5-Amine
Amino-PEG8 Alcohol
477-Peptide
Reserpine
1524-Peptide (2)
iPD1 Peptide
923-Peptide
1524-Peptide
252.1833(1)
118.0876(1)
370.2475(1)
477.2142(1)
762.8842(2)
609.2866(1)
829.5474(1)
923.4309 (1)
1524.7589(1)
Cyclodextrin
Intensity
Mass/Charge

FIG. 6B

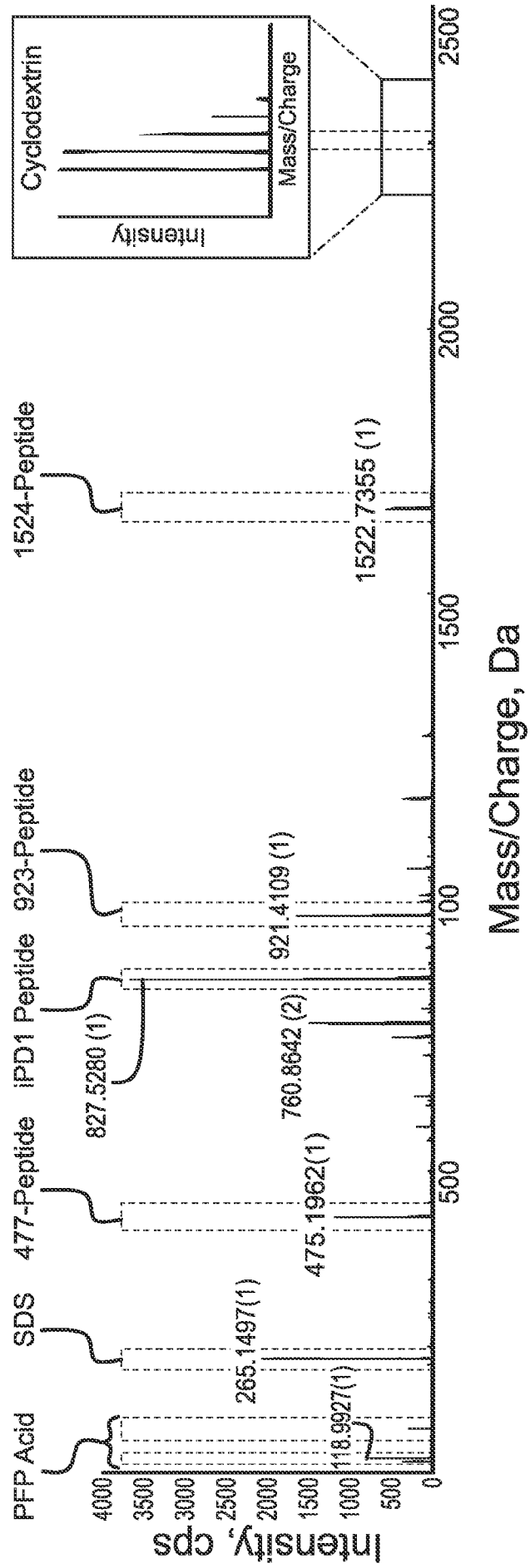

Intensity, cps
Mass/Charge, Da
PFP Acid
SDS
477-Peptide
iPD1 Peptide
923-Peptide
1524-Peptide
118.9927(1)
265.1497(1)
475.1962(1)
827.5280 (1)
760.8642 (2)
921.4109 (1)
1522.7355 (1)
Cyclodextrin
Intensity
Mass/Charge

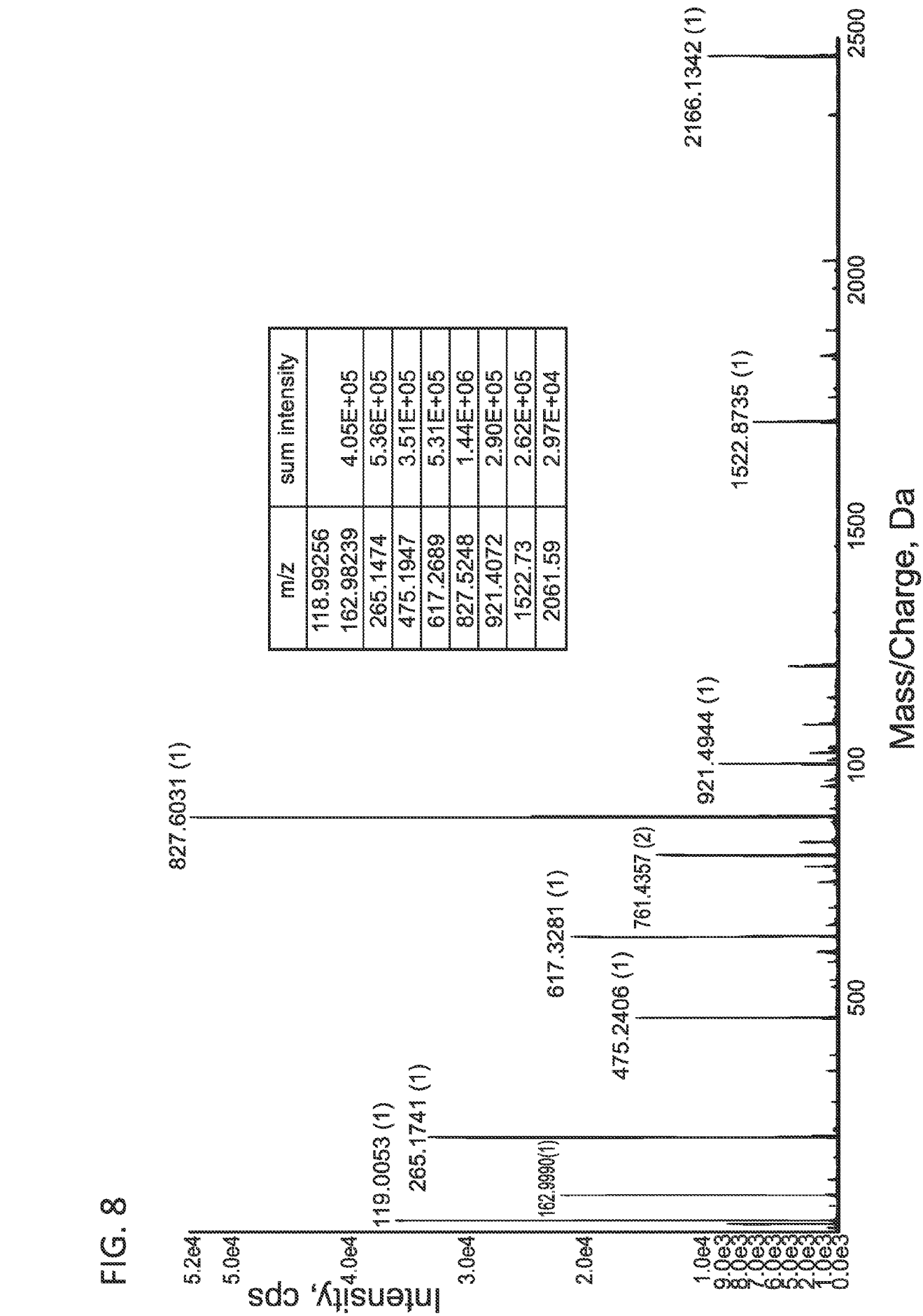
FIG. 7

Table 3. Calibrant Composition 3

Compound	Formula A	Formula B	Formula C	Target m/z	
	Target	Target	Target		
Name	M	M	M	Positive	Negative
Imidazole	1.00E-06	1.00E-06	1.00E-06	N/A	N/A
Betaine	2.00E-05	4.00E-05	2.00E-05	118.0862551	N.D.
Pentafluoropropionic Acid	6.00E-06	1.20E-05	1.20E-05	N/A	118.99256 | 162.98239
m-PEG5-Amine	5.00E-07	5.00E-07	5.00E-07	252.1805494	N.D.
Sodium Dodecyl Sulfate	1.00E-06	1.00E-06	1.00E-06	N.D.	265.1473555
Amino-PEG8-Alcohol	2.50E-07	2.50E-07	2.50E-07	370.2435436	N.D.
H2N-GQWS-OH (SEQ ID NO: 2)	4.00E-06	4.00E-06	4.00E-06	477.2092237	475.1946708
H2N-AQGAWS-OH (SEQ ID NO: 3)	2.00E-06	2.00E-06	2.00E-06	619.2834513	617.2688984
H2N-GQGQFGSLQGFSVGVG-OH (SEQ ID NO: 10)	2.00E-06	2.00E-06	2.00E-06	1524.744132	1522.72958
iPD1 Peptide	2.00E-06	2.00E-06	3.00E-06	829.5393316	827.5247787
H2N-AGQSNFSNV-OH (SEQ ID NO: 7)	1.00E-06	1.00E-06	1.00E-06	923.4217357	921.4071828
Triacetyl-β-cyclodextrin*	5.00E-07	5.00E-07	5.00E-07	2034.625448	2061.589825

* + adduct is NH3; - adduct is formate CH2O2

FIG. 8



m/z	sum intensity
118.99256 162.98239	4.05E+05
265.1474	5.36E+05
475.1947	3.51E+05
617.2689	5.31E+05
827.5248	1.44E+06
921.4072	2.90E+05
1522.73	2.62E+05
2061.59	2.97E+04