CLAIMS

We claim:

1. A method of evaluating performance of a mass spectrometer instrument comprising:

providing a mass spectrometer (MS) instrument;

introducing a standard to the MS instrument, wherein the standard comprises an oligonucleotide having a structure of Formula I, Formula II, Formula III, or Formula IV:

$X^1$3′-PL-3′X^2 (I)

$X^1$5′-PL-5′X^2 (II)

$X^1$3′-PL-5′X^2 (III)

$X^1$5′-PL-3′X^2 (IV)

wherein

PL is a phosphate linkage comprising -PO_4-, -PO_3-O-PO_3-, -PO_3S, -PO_3BH_2- or -PO_3-O-PO_2-O-PO_3-;

X^1 and X^2 are independently a nucleoside, a nucleotide, or an oligonucleotide; and

evaluating one or more mass spectrometry signals corresponding to the oligonucleotide from the mass spectrometry instrument.

2. The method of claim 1,

(i) wherein the evaluating comprises identifying one or more mass peaks corresponding to the oligonucleotide, and comparing the identified one or more mass peaks to one or more expected mass peaks for the oligonucleotide;

(ii) wherein the method further comprises adjusting the MS instrument based on the evaluating of the one or more mass spectrometry signals; or

(iii) wherein the mass spectrometry instrument has a negative ionization mode, and wherein the standard is introduced for a negative mode mass spectrometry analysis.

3. The method of claim 2, under (iii), further comprising performing a qualification of a negative ionization mode of the MS instrument,

wherein preferably the qualification comprises:

analyzing the oligonucleotide in a plurality of amounts; and

determining whether the one or more mass spectrometry signals are substantially linear relative to the plurality of amounts,

wherein more preferably the analyzing is a non-linear regression analysis relative to the plurality of amounts used to calibrate or qualify a mass spectrometer.

4. The method of claim 1,

(i) wherein the method further comprises performing a mass-axis calibration;

(ii) wherein the standard is a continuous reference standard introduced to the MS instrument on a continuous basis during sample analysis;

(iii) wherein the standard is an internal standard introduced to the MS instrument simultaneously with a sample for analysis;

(iv) wherein X^1 and X^2 are the same nucleoside;

(v) wherein X^1 and X^2 are different nucleosides;

(vi) wherein the oligonucleotide of Formula I is deoxythymidine-3′-PL-3′-deoxythymidine;

(vii) wherein the oligonucleotide is in solution;

(viii) wherein the oligonucleotide is solubilized from a solid;

(ix) wherein the oligonucleotide is from 2 to 100 nucleobases in length;

(x) wherein the oligonucleotide is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length;

(xi) wherein the oligonucleotide is 25-30, 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 95-100 nucleobases in length;

(xii) wherein the oligonucleotide comprises MOE, 2'fluoro, 2'Ome, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5 -propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 6-O-methylguanine, LNA, UNA, C(et), 2-thiocytidine, methylated bases, intercalated bases, a nucleotide comprising a modified sugar (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose), or any combination thereof;

(xiii) wherein the oligonucleotide further comprises TBDMS, MOM, MEM, DMT, TOM, ACE, TC, PMB, Methylthiomethyl phosphate, ether, Pivaloyl, ether, silyl ether, methyl ester, and trityl; or

(xiv) wherein the oligonucleotide further comprises an alkyl spacer.

5. The method of claim 4, under (xiv), wherein the alkyl spacer is selected from dodecyl, undecyl, decyl, nonyl, octyl, heptyl, hexyl, pentyl, butyl, propyl, ethyl, and methyl.

6. The method of claim 1,

(i) wherein the oligonucleotide further comprises a GalNAc and/or PEG residue; or

(ii) wherein the oligonucleotide further comprises a 2', 3', and/or 5' synthesis protecting group.

7. The method of claim 6, under (ii), wherein the 2', 3' an/or 5' synthesis protecting group is selected from TBDMS, MOM, MEM, TOM, ACE, TC, DMT, PMB, Methylthiomethyl ether, Pivaloyl, ether, silyl ether, methyl ester, and trityl.

8. The method of claim 1,

(i) wherein the oligonucleotide is composed of 3' and/or 5' linkages; or

(ii) wherein the oligonucleotide comprises a synthesis protecting group.

9. The method of claim 8, under (ii), wherein the synthesis protecting group is selected from the group consisting of acetyl, isobutyl, TAC, dmf, and benzoyl.

10. The method of claim 1,

(i) wherein the oligonucleotide comprises a phosphate with a CNET protecting group; or

(ii) wherein the method further comprises calibrating, standardizing, or qualifying an MS/MS mode of the MS instrument using the evaluated one or more mass spectrometry signals.

11. The method of claim 10, under (ii),

(i) wherein the standard is used to calibrate the MS/MS mode of a mass spectrometer;

(ii) wherein the standard is used to standardize the MS/MS mode of a mass spectrometer;

(iii) wherein the standard is used to qualify the MS/MS mode of a mass spectrometer; or

(iv) wherein the standard is infused into the MS instrument with a sample, and a known mass of the standard is used to dynamically or continuously correct or adjust the MS instrument's m/z calibration.

12. The method of claim 1,

(i) wherein the method further comprises loading the standard into a standard vessel; and the standard is introduced to the MS instrument by flowing from the standard vessel, wherein preferably the standard is continuously flowed to the MS instrument for a selected or predetermined period; or

(ii) wherein the method further comprises fluidically connecting the MS instrument to an oligonucleotide synthesis reaction chamber, and receiving a sample from the oligonucleotide synthesis reaction chamber.

13. The method of claim 12, under (ii),

(i) wherein the standard is introduced to the MS instrument on a continuous basis during analysis of the sample from the oligonucleotide synthesis reaction chamber;

(ii) wherein the sample from the oligonucleotide synthesis reaction chamber comprises an oligonucleotide comprising a synthesis protecting group,

wherein preferably the synthesis protecting group is selected from acetyl, isobutyl, TAC, dmf, benzoyl, TBDMS, MOM, MEM, DMT, PMB, Methylthiomethyl ether, Pivaloyl, ether, silyl ether, methyl ester, and trityl;

(iii) wherein the sample from the oligonucleotide synthesis reaction chamber comprises an oligonucleotide comprising a phosphate with a CNET protecting group; or

(iv) wherein the sample from the oligonucleotide synthesis reaction chamber comprises an oligonucleotide comprising a GalNAc and/or PEG residue.

14. The method of claim 1, wherein the oligonucleotide of the standard comprises a plurality of oligonucleotides having a structure of Formula I, II, III, or IV.

15. The method of claim 14,

(i) wherein the two or more oligonucleotides have different molecular weights; or

(ii) wherein the two or more oligonucleotides have different lengths.