

CLAIMS

What is claimed is:

1. A method of identifying a test ampholyte composition with suitable activity, comprising:
 - a. identifying at least one marker in at least one test ampholyte composition and in a reference ampholyte composition using liquid chromatography-mass spectrometry (LC-MS); and
 - b. determining the degree of similarity or difference of the at least one marker between the at least one test ampholyte composition and the reference ampholyte composition,
 - wherein the at least one test ampholyte composition has suitable activity if the at least one marker has a low covariance and the level of the at least one marker is different between the at least one test ampholyte composition and the reference ampholyte composition,
 - or wherein the at least one test ampholyte composition has suitable activity if the at least one marker has a high covariance and the level of the at least one marker is similar between the at least one test ampholyte composition and the reference ampholyte composition;thereby identifying a test ampholyte composition with suitable activity.
2. The method of claim 1, wherein step (a) comprises
 - i. determining Accurate Mass/Retention Time (AMRT) and/or collision cross section measurements for a plurality of components of the at least one test ampholyte composition and the reference ampholyte composition;
 - ii. plotting covariance of the AMRT and/or collision cross section measurements for the plurality of components from the at least one test and reference ampholyte compositions using an S-plot; and
 - iii. selecting at least one component that is different between the at least one test ampholyte composition and the reference ampholyte composition, wherein the difference comprises a covariance that is non-0 in the S-plot;

thereby identifying at least one marker for characterizing the suitability of the at least one test ampholyte composition.

3. The method of claim 2, wherein the at least one component comprises a covariance in the S-plot that is less than the covariance of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% of the plurality of components in the S-plot.
4. The method of claim 2 or 3, wherein the at least one test ampholyte composition has suitable activity if the level of the at least one marker in the at least one test ampholyte composition and the reference ampholyte composition is different.
5. The method of claim 4, wherein the difference in the level of the at least one marker comprises a difference in level of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% relative to a normalized level of the at least one marker.
6. The method of claim 1, wherein step (a) comprises
 - i. determining Accurate Mass/Retention Time (AMRT) or collision cross section measurements for a plurality of components of the at least one test ampholyte composition and the reference ampholyte composition;
 - ii. plotting covariance of the AMRT or collision cross section measurements for the plurality of components from the at least one test and reference ampholyte compositions using an S-plot; and
 - iii. selecting at least one component that is similar between the at least one test ampholyte composition and the reference ampholyte composition, wherein the similarity comprises a covariance that is non-0 in the S-plot;
thereby identifying at least one marker for characterizing the suitability of the at least one test ampholyte composition.
7. The method of claim 6, wherein the at least one component comprises a covariance in the S-plot that is greater than the covariance of at least 40%, at least 50%, at least

- 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% of the plurality of components in the S-plot.
8. The method of claim 6 or 7, wherein the at least one test ampholyte composition has suitable activity if the level of the at least one marker in the at least one test ampholyte composition and the reference ampholyte composition is similar.
 9. The method of claim 8, wherein the similarity in the level of the at least one marker comprises a level that is at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 99% similar relative to a normalized level of the at least one marker.
 10. The method of any one of claims 1-9, wherein the at least one marker is identified by a mass to charge ratio (m/z).
 11. The method of claim 10, wherein the level of the at least one marker is characterized by relative intensity of the m/z in a mass spectrum.
 12. The method of claim 11, wherein step (b) comprises:
 - i. determining a LC-MS mass spectrum of the at least one marker in the at least one test ampholyte composition and the reference ampholyte composition,
 - ii. determining the relative intensity of a base peak for the at least one marker in the mass spectrums of the at least one test ampholyte composition and the reference ampholyte composition,
 - iii. normalizing the relative intensity of the base peak of the at least one marker to a maximum relative intensity of the base peak measured from the at least one test ampholyte composition or the reference ampholyte composition, and
 - iv. comparing the normalized relative intensities of the base peak of the at least one marker in the at least one test and reference ampholyte compositions.

13. The method of any one of claims 1-12, wherein the at least one marker comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 markers with different m/z .
14. The method of any one of claims 1-12, wherein the at least one marker comprises 16 markers with different m/z .
15. The method of claim 14, wherein the 16 markers comprise markers with an m/z of 280, an m/z of 319, an m/z of 329, an m/z of 347, an m/z of 373, an m/z of 375, an m/z of 376, an m/z of 431, an m/z of 504, an m/z of 506, an m/z of 508, an m/z of 520, an m/z of 534, an m/z of 562, an m/z of 906 and an m/z of 980.
16. The method of any one of claims 1-15, wherein the mass spectrometry comprises ion mobility Quadrupole Time-of-flight Mass Spectrometry (IMS-Q-ToF-MS).
17. The method of any one of claims 1-16, wherein the liquid chromatography comprises high-performance liquid chromatography (HPLC).
18. The method of claim 17, wherein the HPLC comprises a C4 silica column.
19. The method of any one of claims 1-18, further comprising validating the at least one test ampholyte composition by generating an imaged capillary isoelectric focusing (iCIEF) electropherogram of a reference protein using the reference ampholyte composition and the at least one test ampholyte composition, thereby generating at least one test electropherogram and a reference electropherogram.
20. The method of claim 19, wherein the test ampholyte composition is validated if the at least one test and the reference electropherograms are similar.
21. The method of claim 20, wherein the similarity of the at least one test and reference electropherograms is determined by number, size, or isoelectric point (pI) of peaks, or a combination thereof.
22. The method of claim 20 or 21, wherein the similarity of the at least one test and

reference electropherograms comprises having an identical number of peaks.

23. The method of any one of claims 20-22, wherein the similarity of the at least one test and reference electropherograms comprises a difference in an area under the peak of a region of the reference and at least one test electropherograms with a p-value of less than 0.05.
24. The method of claim 23, wherein the region comprises a major reference peak, a region that is more acidic than the major reference peak, or a region that is more basic than the major reference peak.
25. The method of any one of claims 1-24, wherein the suitable activity comprises capillary isoelectric focusing (CIEF) or imaged capillary isoelectric focusing (iCIEF).
26. The method of claim 25, wherein the iCIEF is used to characterize a protein drug product or drug substance.