CLAIMS

1. A method for analytically separating two or more buffers or excipients in a single assay, the method comprising:

   performing chromatography on a test sample, the sample comprising the two or more buffers or excipients, on a pentafluorophenyl (PFP) high performance liquid chromatography (HPLC) column to separate the two or more buffers or excipients;

   detecting the two or more separated buffers or excipients in the HPLC column effluent; and

   generating a chromatogram having peaks corresponding to the separated two or more buffers or excipients.

2. The method according to claim 1, wherein the two or more buffers or excipients are selected from the group consisting of 2-hydroxypropyl-beta-cyclodextrin, sucrose, sodium phosphate, sodium citrate, potassium phosphate, histidine, trehalose, and mannitol.

3. The method according to claim 1 or 2, wherein the two or more buffers or excipients are sugars or sugar-based molecules.

4. The method according to claim 3, wherein the two or more buffers or excipients are sugars.

5. The method according to claim 3, wherein the two or more buffers or excipients are selected from the group consisting of 2-hydroxypropyl-beta-cyclodextrin, sucrose, trehalose, and mannitol.

6. The method according to any one of claims 1 to 5, further comprising:

   obtaining standard calibration chromatographic data for the two or more excipients run on the same HPLC column; and

   calculating a concentration or an amount of the two or more buffers or excipients in the test sample by determining from the chromatogram integrated peak areas of the two or more buffers or excipients and converting the integrated areas to a concentration or amount based on the obtained standard calibration chromatographic data.

7. The method according to claim 6, wherein the conversion includes a linear regression fit to the standard calibration chromatographic data.

8. The method according to any one of claims 1 to 7, wherein the two or more buffers or excipients are detected using an evaporative light scattering detector (ELSD).

9. The method according to claim 8, wherein the ELSD is set at an evaporative temperature of 40 to 70 °C, a pressure of 30 to 70 psi, a gain of 0.5 to 2, and filter set at 0.5 to 1.

10. The method according to any one of claims 1 to 7, wherein the two or more buffers or excipients are detected using a charged aerosol detector (CAD).

11. The method according to claim 10, wherein the CAD is set at an evaporative temperature of 25 to 35 °C, a frequency of 4 to 6 Hz, a filter set at 4 to 6 seconds, a power function set to 1.78 for the first two-thirds of an HPLC run, and a power function set to 1.68 for the last third of the HPLC run.

12. The method according to any one of claims 1 to 11, wherein the HPLC is run using mobile phase A and mobile phase B.

13. The method according to claim 12, wherein mobile phase A is selected from the group consisting of 100% H_2O, formic acid in H_2O, and trifluoroacetic acid in H_2O.

14. The method according to claim 13, wherein mobile phase A is selected from the group consisting of 0.5% formic acid in H_2O and 0.05% trifluoroacetic acid in H_2O.

15. The method according to any one of claims 12 to 14, wherein mobile phase B comprises acetonitrile.

16. The method according to claim 15, wherein mobile phase B is 100% acetonitrile.

17. The method according to any one of claims 12 to 15, wherein performing chromatography comprises an equilibration step having a flow of 100% mobile phase A through the HPLC column at a rate of 0.1 ml/minute to 1.0 ml/minute.

18. The method according to claim 17, wherein the equilibration step flow rate is 0.25 ml/minute.

19. The method according to claim 17, wherein the equilibration step flow rate is 0.5 ml/minute.

20. The method according to any one of claims 17 to 19, wherein the equilibration step is between 0.5 minutes and 10 minutes.

21. The method according to claim 20, wherein the equilibration step is 3.0 minutes.

22. The method according to claim 20, wherein the equilibration step is 4.0 minutes.

23. The method according to any one of claims 12 to 22, wherein performing chromatography comprises a gradient change step flow of 60% mobile phase A and 40% mobile phase B through the HPLC column.

24. The method according to any one of claims 12 to 22, wherein performing chromatography comprises a gradient change step flow of 40% mobile phase A and 60% mobile phase B through the HPLC column.

25. The method according to any one of claims 12 to 22, wherein performing chromatography comprises a gradient change step flow of 100% mobile phase A through the HPLC column.

26. The method according to any one of claims 23 to 25, wherein the gradient change step flow rate is between 0.1 ml/minute to 1.0 ml/minute.

27. The method according to claim 26, wherein the gradient change step flow rate is 0.25 ml/minute.

28. The method according to claim 26, wherein the gradient change step flow rate is 0.5 ml/minute.

29. The method according to any one of claims 23 to 28, wherein the gradient change step is between 0.5 minutes and 10 minutes.

30. The method according to claim 29, wherein the gradient change step is 0.5 minutes.

31. The method according to claim 29, wherein the gradient change step is 2.0 minutes.

32. The method according to claim 29, wherein the gradient change step is 4.0 minutes.

33. The method according to any one of claims 12 to 32, wherein performing chromatography comprises a maintenance step flow of 40% mobile phase A and 60% mobile phase B through the HPLC column.

34. The method according to any one of claims 12 to 32, wherein performing chromatography comprises a maintenance step flow of 100% mobile phase A through the HPLC column.

35. The method according to claim 33 or 34, wherein the maintenance step flow rate is between 0.1 ml/minute and 1.0 ml/minute.

36. The method according to claim 35, wherein the maintenance step flow rate is 0.5 ml/minute.

37. The method according to claim 35, wherein the maintenance step flow rate is 1.0 ml/minute.

38. The method according to any one of claims 33 to 37, wherein the maintenance step is between 0.5 minutes and 10 minutes.

39. The method according to claim 38, wherein the maintenance step is 2.5 minutes.

40. The method according to claim 38, wherein the maintenance step is 4.0 minutes.

41. The method according to any one of claims 12 to 40, wherein performing chromatography comprises a re-equilibration step having a flow of 100% mobile phase A through the HPLC column at a rate of 0.1 ml/minute to 1.0 ml/minute.

42. The method according to claim 41, wherein the re-equilibration step flow rate is 0.25 ml/minute.

43. The method according to claim 41 or 42, wherein the re-equilibration step is between 0.5 minutes and 10 minutes.

44. The method according to claim 43, wherein the re-equilibration step is 3.0 minutes.

45. The method according to any one of claims 12 to 15, wherein performing chromatography comprises: (i) equilibration with 100% mobile phase A at a flow rate of 0.25 ml/minute for 3.0 minutes; (ii) gradient change to 60% mobile phase A and 40% mobile phase B at a flow rate of 0.25 ml/minute for 0.5 minutes; (iii) gradient change to 40% mobile phase A and 60% mobile phase B at a flow rate of 1.0 ml/minute for 4.0 minutes; (iv) maintenance at 40% mobile phase A and 60% mobile phase B at a flow rate of 1.0 ml/minute for 2.5 minutes; (v) gradient change to 100% mobile phase A at a flow rate of 1.0 ml/minute for 2 minutes; and (vi) re-equilibration with 100% mobile phase A at a flow rate of 0.25 ml/minute for 3.0 minutes.

46. The method according to any one of claims 12 to 15, wherein performing chromatography comprises: (i) equilibration with 100% mobile phase A at a flow rate of 0.5 ml/minute for 4.0 minutes; (ii) gradient change to 40% mobile phase A and 60%

mobile phase B at a flow rate of 0.5 ml/minute for 2.0 minutes; (iii) gradient change to 100% mobile phase A at a flow rate of 0.5 ml/minute for 2.0 minutes; and (iv) maintenance at 100% mobile phase A at a flow rate of 0.5 ml/minute for 4 minutes.

47. The method according to any one of claims 12 to 15, wherein performing chromatography comprises: (i) equilibration with 100% mobile phase A at a flow rate of 0.5 ml/minute for 4.0 minutes; (ii) gradient change to 40% mobile phase A and 60% mobile phase B at a flow rate of 0.5 ml/minute for 2.0 minutes; (iii) gradient change to 100% mobile phase A at a flow rate of 0.5 ml/minute for 2.0 minutes; and (iv) maintenance at 100% mobile phase A at a flow rate of 0.5 ml/minute for 4 minutes.

48. The method according to any one of claims 1 to 47, wherein 1 µl to 100 µl of the test sample is injected into the HPLC column.

49. The method according to claim 48, wherein 10 µl of the test sample is injected into the HPLC column.

50. The method according to claim 48, wherein 4 µl of the test sample is injected into the HPLC column.

51. The method according to any one of claims 1 to 50, wherein the PFP HPLC column is a 2.6 µm 150 x 4.6 mm column.

CLAIMS

1. A method for analytically separating two or more buffers or excipients in a single assay, the method comprising:

   performing chromatography on a test sample, the sample comprising the two or more buffers or excipients, on a pentafluorophenyl (PFP) high performance liquid chromatography (HPLC) column to separate the two or more buffers or excipients;

   detecting the two or more separated buffers or excipients in the HPLC column effluent; and

   generating a chromatogram having peaks corresponding to the separated two or more buffers or excipients.

2. The method according to claim 1, wherein the two or more buffers or excipients are selected from the group consisting of 2-hydroxypropyl-beta-cyclodextrin, sucrose, sodium phosphate, sodium citrate, potassium phosphate, histidine, trehalose, and mannitol.

3. The method according to claim 1 or 2, wherein the two or more buffers or excipients are sugars or sugar-based molecules.

4. The method according to claim 3, wherein the two or more buffers or excipients are sugars.

5. The method according to claim 3, wherein the two or more buffers or excipients are selected from the group consisting of 2-hydroxypropyl-beta-cyclodextrin, sucrose, trehalose, and mannitol.

6. The method according to any one of claims 1 to 5, further comprising:

   obtaining standard calibration chromatographic data for the two or more excipients run on the same HPLC column; and

   calculating a concentration or an amount of the two or more buffers or excipients in the test sample by determining from the chromatogram integrated peak areas of the two or more buffers or excipients and converting the integrated areas to a concentration or amount based on the obtained standard calibration chromatographic data.

7. The method according to claim 6, wherein the conversion includes a linear regression fit to the standard calibration chromatographic data.

8. The method according to any one of claims 1 to 7, wherein the two or more buffers or excipients are detected using an evaporative light scattering detector (ELSD).

9. The method according to claim 8, wherein the ELSD is set at an evaporative temperature of 40 to 70 °C, a pressure of 30 to 70 psi, a gain of 0.5 to 2, and filter set at 0.5 to 1.

10. The method according to any one of claims 1 to 7, wherein the two or more buffers or excipients are detected using a charged aerosol detector (CAD).

11. The method according to claim 10, wherein the CAD is set at an evaporative temperature of 25 to 35 °C, a frequency of 4 to 6 Hz, a filter set at 4 to 6 seconds, a power function set to 1.78 for the first two-thirds of an HPLC run, and a power function set to 1.68 for the last third of the HPLC run.

12. The method according to any one of claims 1 to 11, wherein the HPLC is run using mobile phase A and mobile phase B.

13. The method according to claim 12, wherein mobile phase A is selected from the group consisting of 100% H_2O, formic acid in H_2O, and trifluoroacetic acid in H_2O.

14. The method according to claim 13, wherein mobile phase A is selected from the group consisting of 0.5% formic acid in H_2O and 0.05% trifluoroacetic acid in H_2O.

15. The method according to any one of claims 12 to 14, wherein mobile phase B comprises acetonitrile.

16. The method according to claim 15, wherein mobile phase B is 100% acetonitrile.

17. The method according to any one of claims 12 to 15, wherein performing chromatography comprises an equilibration step having a flow of 100% mobile phase A through the HPLC column at a rate of 0.1 ml/minute to 1.0 ml/minute.

18. The method according to claim 17, wherein the equilibration step flow rate is 0.25 ml/minute.

19. The method according to claim 17, wherein the equilibration step flow rate is 0.5 ml/minute.

20. The method according to any one of claims 17 to 19, wherein the equilibration step is between 0.5 minutes and 10 minutes.

21. The method according to claim 20, wherein the equilibration step is 3.0 minutes.

22. The method according to claim 20, wherein the equilibration step is 4.0 minutes.

23. The method according to any one of claims 12 to 15, wherein performing chromatography comprises: (i) equilibration with 100% mobile phase A at a flow rate of 0.25 ml/minute for 3.0 minutes; (ii) gradient change to 60% mobile phase A and 40% mobile phase B at a flow rate of 0.25 ml/minute for 0.5 minutes; (iii) gradient change to 40% mobile phase A and 60% mobile phase B at a flow rate of 1.0 ml/minute for 4.0 minutes; (iv) maintenance at 40% mobile phase A and 60% mobile phase B at a flow rate of 1.0 ml/minute for 2.5 minutes; (v) gradient change to 100% mobile phase A at a flow rate of 1.0 ml/minute for 2 minutes; and (vi) re-equilibration with 100% mobile phase A at a flow rate of 0.25 ml/minute for 3.0 minutes.

24. The method according to any one of claims 12 to 15, wherein performing chromatography comprises: (i) equilibration with 100% mobile phase A at a flow rate of 0.5 ml/minute for 4.0 minutes; (ii) gradient change to 40% mobile phase A and 60% mobile phase B at a flow rate of 0.5 ml/minute for 2.0 minutes; (iii) gradient change to 100% mobile phase A at a flow rate of 0.5 ml/minute for 2.0 minutes; and (iv) maintenance at 100% mobile phase A at a flow rate of 0.5 ml/minute for 4 minutes.

25. The method according to any one of claims 12 to 15, wherein performing chromatography comprises: (i) equilibration with 100% mobile phase A at a flow rate of 0.5 ml/minute for 4.0 minutes; (ii) gradient change to 40% mobile phase A and 60% mobile phase B at a flow rate of 0.5 ml/minute for 2.0 minutes; (iii) gradient change to 100% mobile phase A at a flow rate of 0.5 ml/minute for 2.0 minutes; and (iv) maintenance at 100% mobile phase A at a flow rate of 0.5 ml/minute for 4 minutes.

26. The method according to any one of claims 1 to 25, wherein 1 µl to 100 µl of the test sample is injected into the HPLC column.

27. The method according to claim 26, wherein 10 µl of the test sample is injected into the HPLC column.

28. The method according to claim 26, wherein 4 µl of the test sample is injected into the HPLC column.

29. The method according to any one of claims 1 to 28, wherein the PFP HPLC column is a 2.6 µm 150 x 4.6 mm column.