CLAIMS

What is claimed:

1. A method for ~~predicting or~~ detecting a column malfunction in a chromatography process, comprising:

(a) contacting a reference sample comprising a plurality of analytes and a biologic composition with a chromatography column comprising a separation composition comprising a matrix, under conditions sufficient for the biologic composition to reversibly bind to and be eluted from the matrix upon contacting the matrix with an elution composition, whereby generating a reference chromatogram from the chromatography column by plotting an absorbance value of at least a first waste composition, a second waste composition and an eluted analyte composition as a function of volume of liquid that traverses the chromatography column, wherein the plotting occurs continuously as the compositions exit the chromatography column;

(b) contacting a process sample comprising the plurality of analytes and the biologic composition with a chromatography column comprising a separation composition comprising a matrix, under conditions sufficient for the biologic composition to reversibly bind to and be eluted from the matrix upon contacting the matrix with the elution composition, whereby generating a process chromatogram from the chromatography column by plotting an absorbance value of at least a first waste composition as a function of volume of liquid that traverses the chromatography column, wherein the plotting occurs continuously as the composition exits the chromatography column;

(c) comparing the process chromatogram to the reference chromatogram thereby detecting an atypical profile in the process chromatogram, wherein the atypical profile comprises one or more features which indicate the column malfunction selected from the group consisting of:

an additional peak on the process chromatogram when compared to a comparable position on the reference chromatogram, and

a measured absorbance intensity exceeding a maximum absorbance intensity during an integral volume window,

wherein the one or more features, which indicate the column malfunction, occur prior to a step of contacting the chromatography column with an elution composition;

(d) generating a warning signal when the atypical profile is detected; and

(e) halting the chromatography process when the atypical profile is detected and until the column malfunction is resolved.

~~(a) generating a reference chromatogram from a chromatography column,~~

~~(b) generating a process chromatogram from the chromatography column, and~~

~~(c) detecting an atypical profile in the process chromatogram,~~

~~wherein the atypical profile indicates a potential or actual malfunction of a chromatography process.~~

~~2. The method of claim 1, wherein the method further comprises (d) generating a warning signal.~~

~~3. The method of claim 2, wherein the method further comprises (e) repacking the chromatography column.~~

4~~2~~. The method of ~~any one of~~ claims ~~1-3~~, wherein the chromatography ~~column is suitable for use~~ process comprises in ion exchange chromatography.

5~~3~~. The method of ~~any one of~~ claims ~~1-3~~, wherein the chromatography ~~column is suitable for use in~~process comprises affinity chromatography.

6~~4~~. The method of ~~any one of~~ claims ~~1-3~~, wherein the chromatography ~~column is suitable for use in~~process comprises hydrophobic interaction chromatography.

7~~5~~. The method of any one of claims 1-4~~6~~, wherein the ~~potential or actual~~column malfunction ~~of the chromatography process~~ is caused by column deterioration.

8~~6~~. The method of claim 5~~7~~, wherein the column deterioration comprises column bed fracturing.

~~9. The method of any one of claims 1-8, wherein the atypical profile comprises an additional peak on the process chromatogram when compared to a comparable position on the reference chromatogram.~~

~~10~~7. The method of any one of claims 1 to ~~6~~9, wherein the additional peak occurs during a washing step in the chromatography process.

~~11. The method of claim 10, wherein the washing step precedes an elution step in the chromatography process.~~

~~12. The method of claim 11, wherein the additional peak occurs during the washing step and precedes the elution step in the chromatography process.~~

~~13~~8. The method of any one of claims ~~9-12~~1 to 7, wherein the magnitude of the additional peak indicates a degree of severity of the ~~potential or actual~~column malfunction ~~of the chromatography process~~.

~~14~~9. The method of claim 8~~13~~, wherein an increase in the magnitude of the additional peak indicates an increase in the degree of severity of the ~~potential or actual~~column malfunction of the chromatography process.

~~15~~10. The method of any one of claims ~~9-14~~1 to 9, wherein the ~~malfunction of the chromatography process~~column malfunction induces a loss of separation power of the chromatography column.

~~16. The method of any one of claims 1-8, wherein the atypical profile comprises at least one peak or at least one indentation on the process chromatogram having a decreased sharpness or a decreased magnitude when compared to a peak or an indentation at a comparable position on the reference chromatogram.~~

~~17. The method of claim 16, wherein the at least one peak or the at least one indentation on the process chromatogram having a decreased sharpness or a decreased magnitude occurs during the washing step.~~

~~18. The method of claim 16 or 17, wherein the at least one peak or the at least one indentation on the process chromatogram having a decreased sharpness or a decreased magnitude follows the elution step.~~

~~19. The method of any one of claims 1-8, wherein the atypical profile comprises a first peak or a first indentation having a decreased sharpness or a decreased magnitude on the process chromatogram when compared to a peak or an indentation at a comparable first position on the reference chromatogram and a second peak or a second indentation having a decreased sharpness or a decreased magnitude on the process chromatogram when compared to a peak or indentation at a comparable second position on the reference chromatogram.~~

~~20. The method of claim 19, wherein the first peak or the first indentation follows the elution step.~~

~~21. The method of claim 20, wherein the first peak or the first indentation corresponds to an indentation occurring at a start of a collecting step.~~

~~22. The method of any one of claims 19-21, wherein the second peak or the second indentation follows a collection step.~~

~~23. The method of claim 22, wherein the second peak or the second indentation comprises a strip peak.~~

~~24. The method of any one of claims 1-23, wherein the process chromatogram comprises a measured absorbance intensity, a maximum absorbance intensity, and an integral volume window.~~

~~25. The method of claim 24, wherein the atypical profile comprises a measured absorbance intensity that exceeds a maximum absorbance intensity during an integral volume window.~~

~~26~~11. The method of any one of claims 1 to 10~~24 or 25~~, wherein the measured absorbance intensity is determined at a wavelength corresponding to ultraviolet (UV) light.

~~27~~12. The method of claim ~~26~~11, wherein the measured absorbance intensity is determined at a wavelength of between 260 nanometers (nm) and 280 nm, inclusive of the endpoints.

~~28~~13. The method of claim ~~26~~11, wherein the measured absorbance intensity is determined at a wavelength of 260 nm, providing an A_{260} value.

~~29~~14. The method of claim ~~26~~11, wherein the measured absorbance intensity is determined at a wavelength of 280 nm, providing an A_{280} value.

~~30~~15. The method of claim ~~28 or 29~~13 or 14, ~~wherein the atypical profile comprises a measured absorbance intensity that exceeds a maximum absorbance intensity during an integral volume window and~~ wherein the maximum absorbance intensity has an A_{260} value or an A_{280} value of at least 0.05.

~~31~~16. The method of any one of claims 1 to 15~~-30~~, wherein ~~a typical profile~~the reference chromatogram comprises a measured absorbance intensity that does not exceed a maximum absorbance intensity during an integral volume window and wherein the maximum absorbance intensity has an A_{260} value or an A_{280} value of 0.05 or less.

~~32~~17. The method of any one of claims 1 to 16~~-31~~, wherein the integral volume window occurs between 1000 L and 2500 L, inclusive of the endpoints.

~~33~~18. The method of claim ~~32~~17, wherein the integral volume window occurs between 1250 L and 2250 L, inclusive of the endpoints.

~~34~~19. The method of claim ~~32~~17, wherein the integral volume window occurs between 1600 L and 1800 L, inclusive of the endpoints.

~~35~~20. The method of any one of claims 1 to 19~~-34~~, wherein the chromatography column comprises an ~~Q Sepharose~~ anion exchange resin.

~~36~~21. The method of any one of claims 1 to 20~~-35~~, wherein the step of (a) generating the reference chromatogram comprises:

(1) binding the biologic composition to the matrix, ~~contacting a starting composition comprising a plurality of pre-determined analytes and a separation composition comprising a matrix, under conditions sufficient for at least one analyte of the starting composition to reversibly bind to the matrix, wherein the chromatography column comprises the separation~~

~~composition,~~ to produce ~~an~~ a biologic composition ~~analyte-bound~~ column and a first waste composition;

(2) contacting the biologic composition bound ~~analyte-bound~~ column of (1) and a washing composition, wherein the biologic composition ~~only the at least one analyte of the starting composition~~ remains bound to the matrix to produce a biologic composition ~~purified analyte~~ bound column and a second waste composition;

(3) contacting the purified biologic composition ~~analyte-bound~~ column of (2) and an elution composition, wherein the biologic composition ~~at least one analyte of the starting composition~~ is released from the matrix into the elution composition to produce an eluted biologic ~~analyte~~ composition;

(4) collecting the eluted biologic~~analyte~~ composition;

(5) simultaneously with each of steps (1)-(4), contacting each of the first waste composition, the second waste composition, and the eluted biologic~~analyte~~ composition with ultraviolet (UV) light,

(6) measuring an absorbance of the UV light of each of the first waste composition, the second waste composition, and the eluted biologic~~analyte~~ composition to produce an absorbance value for each of the first waste composition, the second waste composition, and the eluted biologic~~analyte~~ composition; and

(7) generating a chromatogram by plotting the absorbance value of each of the first waste composition, the second waste composition, and the eluted biologic ~~analyte~~ composition as a function of volume of liquid that traverses the chromatography column.

~~37. The method of any one of claims 1-35, wherein the step of (b) generating the process chromatogram comprises~~

~~(1) contacting a starting composition comprising a plurality of test analytes and a separation composition comprising a matrix, under conditions sufficient for at least one analyte of the starting composition to reversibly bind to the matrix, wherein the chromatography column comprises the separation composition, to produce an analyte-bound column and a first waste composition;~~

~~(2) contacting the analyte-bound column of (1) and a washing composition, wherein only the at least one analyte of the starting composition remains bound to the matrix to produce a purified analyte-bound column and a second waste composition;~~

~~(3) contacting the purified analyte-bound column of (2) and an elution composition, wherein the at least one analyte of the starting composition is released from the matrix into the elution composition to produce an eluted analyte composition;~~

~~(4) collecting the eluted analyte composition;~~

~~(5) simultaneously with each of steps (1)-(4), contacting each of the first waste composition, the second waste composition, and the eluted analyte composition with ultraviolet (UV) light,~~

~~(6) measuring an absorbance of the UV light of each of the first waste composition, the second waste composition, and the eluted analyte composition to produce an absorbance value for each of the first waste composition, the second waste composition, and the eluted analyte composition; and~~

~~(7) generating a chromatogram by plotting the absorbance value of each of the first waste composition, the second waste composition, and the eluted analyte composition as a function of volume of liquid that traverses the chromatography column.~~

~~38~~22. The method of claim ~~36 or 37~~21, wherein the contacting step (5) and measuring step (6) are continuous.

~~39~~23. The method of ~~any one of claims 36-38~~claim 21 or claim 22, wherein the matrix comprises a resin.

~~40. The method of any one of claims 36-38, wherein the test analyte is a biologic composition.~~

~~41~~24. The method of any one of claims 21 to 23~~40~~, wherein the biologic composition is a therapeutic composition for use in a method of treatment of a human subject.

~~42~~25. The method of any one of claims ~~2-41~~1-24, wherein the warning signal is communicated electronically to a processor and wherein the processor is operably linked to the chromatography column.

~~43~~26. The method of claim ~~42~~25, wherein the processor receives the warning signal and interrupts the chromatography process or prevents the initiation of a further chromatography

process until the ~~potential or actual malfunction of the chromatography column~~column malfunction is resolved.

27. The method of any one of claims 1 to 26, wherein resolving the column malfunction comprises repacking the chromatography column.

28. The method of any one of claims 1 to 27, wherein the biologic composition comprises a therapeutic protein.

29. The method of claim 28, wherein the therapeutic protein in the reference sample is the same or substantially similar to the therapeutic protein in the process sample.

30. The method of any one of claims 1 to 29, wherein the biologic composition comprises a VEGF-Trap protein.

31. The method of claim 1, wherein the step of (b) generating the process chromatogram comprises:

(1) binding the biologic composition to the matrix, to produce a biologic composition bound column and a first waste composition;

(2) contacting the biologic composition bound column of (1) and a washing composition, wherein the biologic composition remains bound to the matrix to produce a purified biologic composition bound column and a second waste composition;

(3) contacting the purified biologic composition bound column of (2) and an elution composition, wherein the biologic composition is released from the matrix into the elution composition to produce an eluted biologic composition;

(4) collecting the eluted biologic composition;

(5) simultaneously with each of steps (1)-(4), contacting each of the first waste composition, the second waste composition, and the eluted biologic composition with ultraviolet (UV) light,

(6) measuring an absorbance of the UV light of each of the first waste composition, the second waste composition, and the eluted biologic composition to produce an absorbance value for each of the first waste composition, the second waste composition, and the eluted biologic composition; and

(7) generating a chromatogram by plotting the absorbance value of each of the first waste composition, the second waste composition, and the eluted biologic composition as a function of volume of liquid that traverses the chromatography column

wherein generating the process chromatogram occurs simultaneously with contacting each of the first waste composition, the second waste composition, and the eluted biologic composition with ultraviolet (UV) light, and

wherein the chromatography process is interrupted prior to step (3) upon detection of an atypical profile until the column malfunction is resolved.