sediment and compressing the packing material. Non-Patent Document 1 describes a column packing method in a preparative liquid chromatography.

CITATION LIST

PATENT DOCUMENT

[0003] [Patent Document 1] Japanese Publication No. H10-253609

[Patent Document 2] WO 2015/053276

[Patent Document 3] Japanese Publication No. 2015-508179

NON-PATENT DOCUMENT

[0004] [Non-Patent Document 1] YMC15 Preparative LC System Manual

SUMMARY ~~OF INVENTION~~

TECHNICAL PROBLEM

[0005] There is a need for methods of chromatography that will allow for the separation of similar compounds with similar retention times by increasing peak resolution, symmetry and theoretical plate number.

SOLUTION TO PROBLEM

[0006] The present inventors discovered a method for purifying a compound by column chromatography and a method for optimizing the symmetry factor and theoretical plate number by allowing the slurry to stand and reducing the sedimentation volume of the slurry, and completed the present disclosure~~invention~~. The present ~~invention~~ disclosure is directed to a method of increasing the peak resolution, symmetry and theoretical plate number by allowing the slurry to stand for a predetermined length of time. The specification encompasses the following ~~embodiment~~examples of the ~~invention~~disclosure.

[0006a] in an aspect, there is provided a method of purifying one or more compounds by column chromatography comprising:

adding a stationary phase to a column, and

allowing a slurry comprising the stationary phase to settle in the column for a standing time, wherein the stationary phase is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an

esterified derivative thereof.

[0006b] In another aspect, there is provided a method of packing a chromatography column in column chromatography comprising:

adding a stationary phase to a column, and

allowing a slurry comprising the stationary phase to settle in the column for a standing time, wherein the stationary phase is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof.

[0006c] In another aspect, there is provided a method of increasing theoretical plate number (*N*) in a column chromatography comprising:

adding a stationary phase to a column, and

allowing a slurry comprising the stationary phase to settle in the column for a standing time, wherein the increase in *N* is relative to a column chromatography without standing time or wherein the increase in *N* is relative to a slurry that settles for a reduced standing time that is less than the standing time, wherein the stationary phase is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof.

[0006d] In another aspect, there is provided a method of improving symmetry factor of a chromatographic peak in column chromatography comprising allowing a slurry to settle in the column for a standing time,

wherein the improvement comprises decreasing the symmetry factor of the peak to a quantity $\geq 1$, and wherein the decrease is relative to a symmetry factor when a column chromatography comprises a reduced standing time that is absent or less than the standing time and the symmetry factor is $> 1$; or

the improvement comprises increasing the symmetry factor of the peak to a quantity $\leq 1$, and wherein the increase is relative to a symmetry factor when a the column chromatography comprises a reduced standing time that is absent or less than the standing time and the symmetry factor is $< 1$, and wherein the slurry comprises a stationary phase that

is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof.

[0006e] In another aspect, there is provided a method of purifying one or more compounds by column chromatography comprising decreasing sedimentation volume in a slurry, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry.

[0006f] In another aspect, there is provided a method of packing a chromatography column in column chromatography comprising decreasing sedimentation volume in a slurry, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry.

[0006g] In another aspect, there is provide a method of increasing theoretical plate number ($N$) in a column chromatography comprising decreasing sedimentation volume in a slurry, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the decrease in sedimentation volume is relative to a sedimentation volume of a fresh slurry, and wherein the increase in $N$ is relative to an $N$ of a column chromatography wherein the column is packed with the fresh slurry.

[0006h] In another aspect, there is provided a method of improving symmetry factor of a chromatographic peak in column chromatography comprising decreasing sedimentation volume in a slurry before column packing, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry, wherein the improvement comprises decreasing the symmetry factor of the peak to a quantity

≥ 1 if the symmetry factor for the peak > 1 when column chromatography is performed with a column packed with fresh slurry; or the improvement comprises increasing the symmetry factor of the peak to a quantity ≤ 1 if the symmetry factor for the peak < 1 when column chromatography is performed with a column packed with fresh slurry.

[0006i] In another aspect, there is provided a compound or composition purified by the method as defined above.

[0007] [1-1] A method of purifying one or more compounds by column chromatography comprising:

adding a stationary phase to a column, and

allowing a slurry comprising the stationary phase to settle in the column for a settling time.

[1-2] A method of packing a chromatography column comprising:

than about 99%, equal to or greater than about 99.5%, equal to or greater than about 99.8%, or g equal to or greater than about 99.9%.

[0010] Some embodiments of the present disclosure are directed to methods of purifying one or more compounds by column chromatography comprising: adding a stationary phase to a column, and allowing a slurry comprising the stationary phase to settle in the column for a settling time.

[0011] Some ~~embodiment~~examples of the present disclosure are directed to methods of packing a chromatography column comprising: adding a stationary phase to a column, and allowing a slurry comprising the stationary phase to settle in the column for a settling time.

[0012] Some ~~embodiment~~examples of the present disclosure are directed to methods of increasing theoretical plate number (*N*) in a column chromatography comprising: adding a stationary phase to a column, and allowing a slurry comprising the stationary phase to settle in the column for a settling time, wherein the increase in *N* is relative to a column chromatography without settling time or wherein the increase in *N* is relative to a slurry that settles for a reduced settling time that is less than the settling time.

[0013] Some ~~embodiment~~examples of the present disclosure are directed to methods of improving symmetry factor of a chromatographic peak in column chromatography comprising allowing a slurry to settle in the column for a settling time, wherein the improvement comprises decreasing the symmetry factor of the peak to a quantity $\geq 1$, and wherein the decrease is relative to a symmetry factor when a column chromatography comprises a reduced settling time that is absent or less than the settling time and the symmetry factor is $> 1$; or the improvement comprises increasing the symmetry factor of the peak to a quantity $\leq 1$, and wherein the increase is relative to a symmetry factor when the column chromatography comprises a reduced settling time that is absent or less than the settling time and the symmetry factor is $< 1$. In some ~~embodiment~~examples, all chromatography conditions other than settling time are identical when the column chromatography comprises the settling time and when the column chromatography comprises the reduced settling time that is absent or less than the settling time.

[0014] In some ~~embodiment~~examples of these methods, the column is under atmospheric pressure during settling time. In some ~~embodiment~~examples of these methods, pressure is applied to the column after settling time. In some ~~embodiment~~examples of these methods, each settling time is independently between about 0.5 minutes to about 2 minutes, about 2 minutes to about 4 minutes, about 4 minutes to about 6 minutes, about 6 minutes to about 8 minutes, about 8 minutes to about 10 minutes, about 10 minutes to about 12 minutes, about 12 minutes to about 14 minutes, about 14 minutes to about 16 minutes, about 16 minutes to about 18 minutes, about 18 minutes to about 20 minutes, about 20 minutes to about 22 minutes, about 22 minutes to about 24 minutes, about 24 minutes to about 26 minutes, about 26 minutes to about 28 minutes, about 28 minutes to about 30 minutes, about 30 minutes to about 32 minutes, about 32 minutes to about 34 minutes, about 34 minutes to about 36 minutes, about 36 minutes to about 38 minutes, or about 38 minutes to about 40 minutes. In some ~~embodiment~~examples, the settling time is independently between about 0.5 minutes to about 5 minutes, about 5 minutes to about 10 minutes, about 10 minutes to about 15 minutes, about 15 minutes to about 20 minutes, about 20 minutes to about 25 minutes, about 25 minutes to about 30 minutes, about 30 minutes to about 35 minutes, about 35 minutes to about 40 minutes, about 40 minutes to about 45 minutes, about 45 minutes to about 50 minutes, about 50 minutes to about 55 minutes, about 55 minutes to about 60 minutes, about 60 minutes to about 65 minutes, about 65 minutes to about 70 minutes, about 70 minutes to about 75 minutes, about 75 minutes to about 80 minutes, about 80 minutes to about 85 minutes, about 85 minutes to about 90 minutes, about 90 minutes to about 95 minutes, or about 95 minutes to about 100 minutes, about 100 minutes to about 105 minutes, about 105 minutes to about 110 minutes, about 110 minutes to about 115 minutes, about 115 minutes to about 120 minutes.

[0015] Some ~~embodiment~~examples of the present disclosure are directed to methods of purifying one or more compounds by column chromatography comprising decreasing sedimentation volume in a slurry, wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry.

[0016] Some ~~embodiment~~examples of the present disclosure are directed to methods of packing a chromatography column comprising decreasing sedimentation volume in a slurry, wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry.

[0017] Some ~~embodiment~~examples of the present disclosure are directed to methods of increasing theoretical plate number ($N$) in a column chromatography comprising decreasing sedimentation volume in a slurry, wherein the decrease in sedimentation volume is relative to a sedimentation volume of a fresh slurry, and wherein the increase in $N$ is relative to an $N$ of a column chromatography wherein the column is packed with the fresh slurry.

[0018] Some ~~embodiment~~examples of the present disclosure are directed to methods of improving symmetry factor of a chromatographic peak in column chromatography comprising decreasing sedimentation volume in a slurry before column packing, wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry, wherein the improvement comprises decreasing the symmetry factor of the peak to a quantity $\geq 1$ if the symmetry factor for the peak $> 1$ when column chromatography is performed with a column packed with fresh slurry; or the improvement comprises increasing the symmetry factor of the peak to a quantity $\leq 1$ if the symmetry factor for the peak $< 1$ when column chromatography is performed with a column packed with fresh slurry.

[0019] In some ~~embodiment~~examples of these methods, all chromatography conditions other than sedimentation volume are identical when the column chromatography comprises the decreased sedimentation volume and when the column chromatography is performed with a column packed with fresh slurry. In some ~~embodiment~~examples of these methods, the fresh slurry comprises a mobile phase (or solvent) and a stationary phase combined for more than about 0.5 minutes, 1 minute, 2 minutes, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 70 minutes, 80 minutes, 90 minutes, 100 minutes, 110 minutes, 120 minutes and/or less than 200 minutes, 185 minutes, 175 minutes, 165 minutes, 155 minutes, 145 minutes, 135 minutes, 125 minutes, 115 minutes, 105 minutes, 95 minutes, 85 minutes, 75 minutes, 65 minutes, 55 minutes, 45 minutes, 35 minutes, 25 minutes. Combining the mobile phase (or solvent) and the stationary phase includes agitation. In some ~~embodiment~~examples

of these methods, the decrease in sedimentation volume is over an amount of time between about 0 minutes to about 2 minutes, about 2 minutes to about 4 minutes, about 4 minutes to about 8 minutes, about 8 minutes to about 16 minutes, about 16 minutes to about 24 minutes, about 24 minutes to about 32 minutes, about 32 minutes to about 50 minutes, about 50 minutes to about 70 minutes, about 70 minutes to about 90 minutes, about 90 minutes to about 110 minutes, about 110 minutes to about 130 minutes, about 130 minutes to about 150 minutes. In some ~~embodiment~~examples of these methods, the sedimentation volume remains substantially constant after the amount of time. In some ~~embodiment~~examples of these methods, the decrease in sedimentation volume is over a settling time. In some ~~embodiment~~examples of these methods, wherein decreasing sedimentation volume comprises allowing the mixture to settle at atmospheric pressure. In some ~~embodiment~~examples of these methods, pressure is applied to the column after the decrease in sedimentation volume. In some ~~embodiment~~examples of these methods, the column is an axial compression column. In some ~~embodiment~~examples of these methods, the slurry comprises a stationary phase and one or more of water, methanol, ethanol, acetonitrile, ethyl acetate, hexanes, or dichloromethane. In some ~~embodiment~~examples of these methods, the slurry comprises methanol. In some ~~embodiment~~examples of these methods, the stationary phase comprises particles of C30, C22, C18, C8, C5, C4, biphenyl, fluorophenyl, hydrophilic interaction liquid chromatography (HILIC) stationary phase, acrylamide, silica, phenyl-hexyl stationary phase, polar embedded alkyl, and/or fluorophenyl propyl. In some ~~embodiment~~examples of these methods, the stationary phase is particles of C18.

[0020] The method of any one of the above ~~embodiment~~examples, wherein chromatography comprises elution with a mobile phase comprising one or more of water, methanol, ethanol, acetonitrile, ethyl acetate, hexanes, dichloromethane, or supercritical carbon dioxide. In some ~~embodiment~~examples of these methods, the mobile phase comprises methanol. In some ~~embodiment~~examples of these methods, chromatography comprises a mobile phase comprising an additive selected from one or more of formic acid, ammonium formate, trimethylamine, ammonia, and ammonium hydroxide. In some ~~embodiment~~examples of these methods, column chromatography comprises a mobile phase gradient. In some ~~embodiment~~examples of these

methods, chromatography comprises the purification of one or more analytes selected from fatty acids or esterified derivatives thereof, proteins, nucleic acids, and small molecules. In some ~~embodiment~~examples of these methods, chromatography comprises the purification of one or more fatty acids or an esterified derivative thereof. In some ~~embodiment~~examples of these methods, the fatty acids are selected from one or more of docosahexaenoic acid, crotonic acid, myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, gadoleic acid, eicosenoic acid, erucic acid, nervonic acid, linoleic acid, eicosadienoic acid, docosadienoic acid, linolenic acid, pinolenic acid, eleostearic acid, mead acid, dihomo-γ-linolenic acid, eicosatrienoic acid, stearidonic acid, arachidonic acid, eicosatetraenoic acid, adrenic acid, bosseopentaenoic acid, eicosapentaenoic acid, ozubondo acid, sardine acid, tetracosanolpentaenoic acid, cervonic acid, herring acid, propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, nonadecylic acid, arachidic acid, heneicosylic acid, behenic acid, tricosylic acid, lignoceric acid, pentacosylic acid, cerotic acid, carboceric acid, montanic acid, nonacosylic acid, melissic acid, hentriacontylic acid, lacceroic acid, psyllic acid, geddic acid, ceroplastic acid, hexatriacontylic acid, heptatriacontylic acid, octatriacontylic acid, nonatriacontylic acid, and tetracontylic acid. In some ~~embodiment~~examples of these methods, the esterified derivative thereof is a methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl ester. In some ~~embodiment~~examples of these methods, n chromatography comprises the purification of eicosapentaenoic acid ethyl ester. In some ~~embodiment~~examples of these methods, the slurry is formed by: a) premixing a stationary phase and a mobile phase (or solvent) prior to addition of slurry to the column; or b) adding mobile phase (or solvent) to the column then adding stationary phase to the mobile phase. In some ~~embodiment~~examples of these methods, the stationary phase is added to the mobile phase (or solvent) portion wise.

[0021] Other ~~embodiment~~examples include a compound or composition comprising the compound purified by the methods of this disclosure. In some ~~embodiment~~examples, the compound is a fatty acid or esterified derivative thereof, protein, nucleic acid, or small molecule. In some

~~embodiment~~examples, the compound is a fatty acid or an esterified derivative thereof. In some ~~embodiment~~examples, the compound is docosahexaenoic acid, crotonic acid, myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, gadoleic acid, eicosenoic acid, erucic acid, nervonic acid, linoleic acid, eicosadienoic acid, docosadienoic acid, linolenic acid, pinolenic acid, eleostearic acid, mead acid, dihomo-γ-linolenic acid, eicosatrienoic acid, stearidonic acid, arachidonic acid, eicosatetraenoic acid, adrenic acid, bosseopentaenoic acid, eicosapentaenoic acid, ozubondo acid, sardine acid, tetracosanolpentaenoic acid, cervonic acid, herring acid, propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, nonadecylic acid, arachidic acid, heneicosylic acid, behenic acid, tricosylic acid, lignoceric acid, pentacosylic acid, cerotic acid, carboceric acid, montanic acid, nonacosylic acid, melissic acid, hentriacontylic acid, lacceroic acid, psyllic acid, geddic acid, ceroplastic acid, hexatriacontylic acid, heptatriacontylic acid, octatriacontylic acid, nonatriacontylic acid, tetracontylic acid, or an esterified derivative thereof. In some ~~embodiment~~examples, the esterified derivative thereof is a methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl ester. In some ~~embodiment~~examples, the compound or composition is eicosapentaenoic acid ethyl ester. In some ~~embodiment~~examples, the compound or composition comprising a purity of greater than about 50%, greater than about 60%, greater than about 70%, greater than about 80%, greater than about 85%, greater than about 86%, greater than about 90%, greater than about 95%, greater than about 96.5%, greater than about 98%, greater than about 99%, greater than about 99.5%, greater than about 99.8%, or greater than about 99.9% and/or less than about 99.95%, less than about 99.99%. In some ~~embodiment~~examples, the yield is greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, and/or less than 75%, less than 80%, less than 85%, less than 90%, or less than 95%. In some ~~embodiment~~examples, the recovery rate is greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95%, greater than 98%, greater than 99%, or greater than

CLAIMS

1. A method of purifying one or more compounds by column chromatography comprising:

adding a stationary phase to a column, and

allowing a slurry comprising the stationary phase to settle in the column for a ~~settling time~~standing time, wherein the stationary phase is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof.

2. A method of packing a chromatography column in column chromatography comprising:

adding a stationary phase to a column, and

allowing a slurry comprising the stationary phase to settle in the column for a ~~settling time~~standing time, wherein the stationary phase is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof.

3. A method of increasing theoretical plate number (*N*) in a column chromatography comprising:

adding a stationary phase to a column, and

allowing a slurry comprising the stationary phase to settle in the column for a ~~settling~~standing time, wherein the increase in *N* is relative to a column chromatography without ~~settling~~standing time or wherein the increase in *N* is relative to a slurry that settles for a reduced standing~~settling~~ time that is less than the ~~settling~~standing time, wherein the stationary phase is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof.

4. A method of improving symmetry factor of a chromatographic peak in column chromatography comprising allowing a slurry to settle in the column for a ~~settling~~ standing time,

wherein the improvement comprises decreasing the symmetry factor of the peak to a quantity ≥ 1, and wherein the decrease is relative to a symmetry factor when a column chromatography comprises a reduced ~~settling~~ standing time that is absent or less than the ~~settling~~ standing time and the symmetry factor is > 1; or

the improvement comprises increasing the symmetry factor of the peak to a quantity ≤ 1, and wherein the increase is relative to a symmetry factor when a the column chromatography comprises a reduced ~~settling~~standing time that is absent or less than the ~~settling~~ standing time and the symmetry factor is < 1, and wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof.

5. The method of claim 4, wherein all chromatography conditions other than ~~settling~~ standing time are identical when the column chromatography comprises the ~~settling~~ standing time or when the column chromatography comprises the reduced ~~settling~~ standing time that is absent or less than the standing~~settling~~ time.

6. The method of any one of claims 1-5, wherein the column is under atmospheric pressure during ~~settling~~ standing time.

7. The method of any one of claims 1-6, wherein pressure is applied to the column after ~~settling~~ standing time.

8. The method of any one of claims 1-7, wherein each standing~~settling~~ time is independently between about 0.5 minutes to about 5 minutes, about 5 minutes to about 10 minutes, about 10 minutes to about 15 minutes, about 15 minutes to about 20 minutes, about 20 minutes to about 25 minutes, about 25 minutes to about 30 minutes, about 30 minutes to about 35 minutes, about 35 minutes to about 40 minutes, about 40 minutes to about 45 minutes, about 45 minutes to about 50 minutes, about 50 minutes to about 55 minutes, about 55 minutes to about 60 minutes, about 60 minutes to about 65 minutes, about 65 minutes to about 70 minutes, about 70 minutes to about 75 minutes, about 75 minutes to about 80 minutes, about 80 minutes to about 85 minutes, about 85 minutes to about 90 minutes, about

90 minutes to about 95 minutes, or about 95 minutes to about 100 minutes, about 100 minutes to about 105 minutes, about 105 minutes to about 110 minutes, about 110 minutes to about 115 minutes, about 115 minutes to about 120 minutes.

9. A method of purifying one or more compounds by column chromatography comprising decreasing sedimentation volume in a slurry, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry.

10. A method of packing a chromatography column in column chromatography comprising decreasing sedimentation volume in a slurry, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry.

11. A method of increasing theoretical plate number ($N$) in a column chromatography comprising decreasing sedimentation volume in a slurry, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the decrease in sedimentation volume is relative to a sedimentation volume of a fresh slurry, and wherein the increase in $N$ is relative to an $N$ of a column chromatography wherein the column is packed with the fresh slurry.

12. A method of improving symmetry factor of a chromatographic peak in column chromatography comprising decreasing sedimentation volume in a slurry before column packing, wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and the compound to be purified by column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the decrease in sedimentation volume is relative to the sedimentation volume of a fresh slurry, wherein the

improvement comprises decreasing the symmetry factor of the peak to a quantity ≥ 1 if the symmetry factor for the peak > 1 when column chromatography is performed with a column packed with fresh slurry; or the improvement comprises increasing the symmetry factor of the peak to a quantity ≤ 1 if the symmetry factor for the peak < 1 when column chromatography is performed with a column packed with fresh slurry.

13. The method of claim 12, wherein all chromatography conditions other than sedimentation volume are identical when the column chromatography comprises the decreased sedimentation volume or when the column chromatography is performed with a column packed with fresh slurry.

14. The method of claim 12 or 13, wherein the fresh slurry comprises mobile phase and stationary phase combined for less than about 10 minutes, less than about 8 minutes, less than about 6 minutes, less than about 4 minutes, less than about 2 minutes, or less than about 1 minute.

15. The method of any one of claims 9-14, wherein the decrease in sedimentation volume is over an amount of time between about 0 minutes to about 2 minutes, about 2 minutes to about 4 minutes, about 4 minutes to about 6 minutes, about 6 minutes to about 8 minutes, about 8 minutes to about 10 minutes, about 10 minutes to about 12 minutes, about 12 minutes to about 14 minutes, about 14 minutes to about 16 minutes, about 16 minutes to about 18 minutes, about 18 minutes to about 20 minutes, about 20 minutes to about 22 minutes, about 22 minutes to about 24 minutes, about 24 minutes to about 26 minutes, about 26 minutes to about 28 minutes, about 28 minutes to about 30 minutes, about 30 minutes to about 32 minutes, about 32 minutes to about 34 minutes, about 34 minutes to about 36 minutes, about 36 minutes to about 38 minutes, or about 38 minutes to about 40 minutes.

16. The method of claim 15 wherein the sedimentation volume remains substantially constant after the amount of time.

17. The method of any one of claims 9-16, wherein the decrease in sedimentation volume is over a ~~settling~~ standing time.

18. The method of any one of claims 9-17, wherein decreasing sedimentation volume

comprises allowing the mixture to settle at atmospheric pressure.

19. The method of any one of claims 9-18, wherein pressure is applied to the column after the decrease in sedimentation volume.

20. The method of any one of claims 1-18, wherein the column is an axial compression column.

21. The method of any one of claims 1-20, wherein the slurry comprises a stationary phase and one or more of water, methanol, ethanol, acetonitrile, ethyl acetate, hexanes, or dichloromethane.

22. The method of claim 21, wherein the slurry comprises methanol.

23. The method of claim 21 or 22, wherein the stationary phase comprises an average particle size measured by laser diffraction scattering method of greater than 50 μm, greater than 45 μm, greater than 40 μm, greater than 35 μm, greater than 30 μm, greater than 25 μm, greater than 20 μm, greater than 15 μm, or greater than 10 μm.

~~24. The method of claim 21 or 23, wherein the stationary phase comprises particles selected from at least one of C30, C22, C18, C8, C5, C4, biphenyl, fluorophenyl, hydrophilic interaction liquid chromatography (HILIC) stationary phase, acrylamide, silica, phenyl-hexyl stationary phase, polar embedded alkyl, and fluorophenyl propyl.~~

~~25~~24. The method of any one of claims 21-~~24~~23, wherein the stationary phase comprises particles of C18.

~~26~~25. The method of any one of claims 1-~~25~~24, wherein chromatography comprises elution with a mobile phase comprising one or more of water, methanol, ethanol, acetonitrile, ethyl acetate, hexanes, dichloromethane, or supercritical carbon dioxide.

~~27~~26. The method of claim ~~26~~25, wherein the mobile phase comprises methanol.

~~28~~27. The method of any one of claims 1-~~27~~26, wherein chromatography comprises a mobile phase comprising an additive selected from one or more of formic acid, ammonium formate, trimethylamine, ammonia, and ammonium hydroxide.

~~29~~28. The method of any one of claims 1-~~28~~27, wherein column chromatography comprises a mobile phase gradient.

~~30. The method of any one of claims 1-29, wherein chromatography comprises the purification of one or more analytes selected from fatty acids or esterified derivatives thereof, proteins, nucleic acids, and small molecules.~~

~~31. The method of any one of claims 1-30, wherein chromatography comprises the purification of one or more fatty acids or an esterified derivative thereof.~~

~~32~~29. The method of any one of claim~~s 31~~1 to 28, wherein the unsaturated fatty acids are selected from one or more of docosahexaenoic acid, crotonic acid, myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, gadoleic acid, eicosenoic acid, erucic acid, nervonic acid, linoleic acid, eicosadienoic acid, docosadienoic acid, linolenic acid, pinolenic acid, eleostearic acid, mead acid, dihomo-γ-linolenic acid, eicosatrienoic acid, stearidonic acid, arachidonic acid, eicosatetraenoic acid, adrenic acid, bosseopentaenoic acid, eicosapentaenoic acid, ozubondo acid, sardine acid, tetracosanolpentaenoic acid, cervonic acid, and herring acid~~, propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, nonadecylic acid, arachidic acid, henicosylic acid, behenic acid, tricosylic acid, lignoceric acid, pentacosylic acid, cerotic acid, carboceric acid, montanic acid, nonacosylic acid, melissic acid, hentriacontylic acid, lacceroic acid, psyllic acid, geddic acid, ceroplastic acid, hexatriacontylic acid, heptatriacontylic acid, octatriacontylic acid, nonatriacontylic acid, and tetracontylic acid~~.

30~~33~~. The method of any one of claim~~s 31~~1 ~~or~~to ~~32~~29, wherein the esterified derivative thereof is a methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl ester.

~~34~~31. The method of any one of claims 1-~~33~~30, wherein chromatography comprises the purification of eicosapentaenoic acid ethyl ester and/or docosahexaenoic acid ethyl ester.

~~35~~32. The method of any one of claims 1-31~~34~~, wherein the slurry is formed by:

a) premixing stationary phase and mobile phase prior to addition of slurry to the column; or

b) adding mobile phase to the column then adding stationary phase to the mobile

phase.

~~36~~33. The method of claim 32~~34~~, wherein the stationary phase is added to the mobile phase portion wise.

~~37~~34. A compound or composition purified by the method of any one of claims 1-33~~36~~.

~~38. The compound or composition of claim 37, wherein the compound is a fatty acid or esterified derivative thereof, protein, nucleic acid, or small molecule.~~

~~39. The compound or composition of claim 37 or 38, wherein the compound is a fatty acid or an esterified derivative thereof.~~

~~40~~35. The compound or composition of claim ~~39~~34, wherein the compound is docosahexaenoic acid, crotonic acid, myristoleic acid, palmitoleic acid, sapienic acid, oleic acid, elaidic acid, vaccenic acid, gadoleic acid, eicosenoic acid, erucic acid, nervonic acid, linoleic acid, eicosadienoic acid, docosadienoic acid, linolenic acid, pinolenic acid, eleostearic acid, mead acid, dihomo-γ-linolenic acid, eicosatrienoic acid, stearidonic acid, arachidonic acid, eicosatetraenoic acid, adrenic acid, bosseopentaenoic acid, eicosapentaenoic acid, ozubondo acid, sardine acid, tetracosanolpentaenoic acid, cervonic acid, and herring acid~~, propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, nonadecylic acid, arachidic acid, heneicosylic acid, behenic acid, tricosylic acid, lignoceric acid, pentacosylic acid, cerotic acid, carboceric acid, montanic acid, nonacosylic acid, melissic acid, hentriacontylic acid, lacceroic acid, psyllic acid, geddic acid, ceroplastic acid, hexatriacontylic acid, heptatriacontylic acid, octatriacontylic acid, nonatriacontylic acid, tetracontylic acid~~, or an esterified derivative thereof.

~~41~~36. The compound or composition of claim ~~39~~34 or ~~40~~35, wherein the esterified derivative thereof is a methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl ester.

37~~42~~. The compound or composition of any one of claims ~~37~~34-~~41~~36, wherein the compound is eicosapentaenoic acid ethyl ester and/or docosahexaenoic acid ethyl ester.

3843. The compound or composition of any one of claims 3734-4237, comprising a purity of equal to or greater than about 80%, equal to or greater than about 85%, equal to or greater than about 86%, greater than about 90%, greater than about 95%, equal to or greater than about 96.5%, greater than about 98%, greater than about 99%, greater than about 99.5%, greater than about 99.8%, or greater than about 99.9%.

3944. The compound or composition of any one of claims 3436-4338, comprising a yield of greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, or greater than 80%.

4540. The compound or composition of any one of claims 3634-4339, comprising a recovery rate of greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95%, greater than 98%, greater than 99%, or greater than 99.7%.