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

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. The present 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 examples of the 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 a resulting compound obtained by the column chromatography is an unsaturated fatty acid or an

esterified derivative thereof, and wherein the standing time is about 10 minutes or longer, and wherein the addition of the stationary phase is conducted by:

a) adding slurry to the column after the slurry is formed by premixing the stationary phase with a mobile phase; or

b) adding a mobile phase to the column then adding the stationary phase to the mobile phase in the column.

[0006b] In another aspect, there is provided a method 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 standing time, wherein the stationary phase is a reverse phase silica stationary phase, and wherein the standing time is about 10 minutes or longer, and

wherein the addition of the stationary phase is conducted by:

a) adding the slurry to the column after the slurry is formed by premixing the stationary phase with a mobile phase; or

b) adding a mobile phase to the column then adding the stationary phase to the mobile phase in the column.

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

adding a stationary phase to a column,

allowing a slurry comprising the stationary phase to settle in the column for a standing time,

performing the column chromatography using the column after the slurry is settled,

performing a gas chromatography of a resulting compound obtained by the column chromatography, and

calculating the theoretical plate number (*N*) according to equation (1) below,

$$N = \left(\frac{t_R}{\sigma} \right)^2 \quad (1)$$

wherein σ is a standard deviation and t_R is a retention time, and

wherein the increase in *N* is relative to a column chromatography performed without a standing time or wherein the increase in *N* is relative to a column chromatography performed after a slurry is settled for a reduced standing time that is less than the standing time, wherein the stationary phase is a reverse silica stationary phase, and a resulting compound obtained by the column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the standing time is about 10 minutes or longer, and

wherein the addition of the stationary phase is conducted by:

a) adding the slurry to the column after the slurry is formed by premixing stationary phase with a mobile phase; or

b) adding a mobile phase to the column then adding the stationary phase to the mobile phase in the column.

[0006d] In another aspect, there is provided a method of improving a symmetry factor of a chromatographic peak in a column chromatography comprising:

adding a stationary phase to a column,

allowing a slurry to settle in the column for a standing time,

performing the column chromatography using the column after the slurry is settled,

performing a gas chromatography of a resulting compound obtained by the column chromatography, and

calculating a symmetry factor according to equation (2) below,

$$S = \frac{W_{0.05h}}{2f} \quad (2)$$

wherein $W_{0.5h}$ is a peak full width at half maximum (FWHM) , and f is a peak width of a first half of the chromatographic peak measured as a horizontal distance from a point of beginning of the chromatographic peak to an apex of the chromatographic peak, and

wherein the improvement of the symmetry factor comprises decreasing the symmetry factor of the chromatographic peak to a quantity ≥ 1, and wherein the decrease is relative to a symmetry factor that is of a column chromatography performed without a standing time or performed after a slurry is settled for a reduced standing time that is less than the standing time and that is > 1; or

the improvement comprises increasing the symmetry factor of the chromatographic peak to a quantity ≤ 1, and wherein the increase is relative to a symmetry factor that is of the column chromatography performed without a standing time or relative to a column chromatography performed after a slurry is settled for a reduced standing time that is less than the standing time and that is < 1, and wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and a resulting compound obtained by the column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the standing time is about 10 minutes or longer, and

wherein the addition of the stationary phase is conducted by:

a) adding the slurry to the column after the slurry is formed by premixing the stationary phase with a mobile phase; or

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

[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:

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 standing time, wherein the stationary phase is a reverse phase silica stationary phase, and a resulting compound obtained by the column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the standing time is about 10 minutes or longer, and wherein the addition of the stationary phase is conducted by:

a) adding slurry to the column after the slurry is formed by premixing the stationary phase with a mobile phase; or

b) adding a mobile phase to the column then adding the stationary phase to the mobile phase in the column.

2. A method 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 standing time, wherein the stationary phase is a reverse phase silica stationary phase, and wherein the standing time is about 10 minutes or longer, and

wherein the addition of the stationary phase is conducted by:

a) adding the slurry to the column after the slurry is formed by premixing the stationary phase with a mobile phase; or

b) adding a mobile phase to the column then adding the stationary phase to the mobile phase in the column.

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

adding a stationary phase to a column,

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

standing time,

performing the column chromatography using the column after the slurry is settled,

performing a gas chromatography of a resulting compound obtained by the column chromatography, and

calculating the theoretical plate number (*N*) according to equation (1) below,

$$N = \left(\frac{t_R}{\sigma} \right)^2 \quad (1)$$

wherein σ is a standard deviation and t_R is a retention time, and

wherein the increase in *N* is relative to a column chromatography performed without a standing time or wherein the increase in *N* is relative to a column chromatography performed after a slurry is settled for a reduced standing time that is less than the standing time, wherein the stationary phase is a reverse silica stationary phase, and a resulting compound obtained by the column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the standing time is about 10 minutes or longer, and

wherein the addition of the stationary phase is conducted by:

a) adding the slurry to the column after the slurry is formed by premixing stationary phase with a mobile phase; or

b) adding a mobile phase to the column then adding the stationary phase to the mobile phase in the column.

4. A method of improving a symmetry factor of a chromatographic peak in a column chromatography comprising:

adding a stationary phase to a column,

allowing a slurry to settle in the column for a standing time, performing the column chromatography using the column after the slurry is settled,

performing a gas chromatography of a resulting compound obtained by the column chromatography, and

calculating a symmetry factor according to equation (2) below,

$$S = \frac{W_{0.05h}}{2f} \quad (2)$$

wherein $W_{0.5h}$ is a peak full width at half maximum (FWHM) , and f is a peak width of a first half of the chromatographic peak measured as a horizontal distance from a point of beginning of the chromatographic peak to an apex of the chromatographic peak, and

wherein the improvement of the symmetry factor comprises decreasing the symmetry factor of the chromatographic peak to a quantity ≥ 1, and wherein the decrease is relative to a symmetry factor that is of a column chromatography performed without a standing time or performed after a slurry is settled for a reduced standing time that is less than the standing time and that is > 1; or

the improvement comprises increasing the symmetry factor of the chromatographic peak to a quantity ≤ 1, and wherein the increase is relative to a symmetry factor that is of the column chromatography performed without a standing time or relative to a column chromatography performed after a slurry is settled for a reduced standing time that is less than the standing time and that is < 1,

and wherein the slurry comprises a stationary phase that is a reverse phase silica stationary phase, and a resulting compound obtained by the column chromatography is an unsaturated fatty acid or an esterified derivative thereof, and wherein the standing time is about 10 minutes or longer, and

wherein the addition of the stationary phase is conducted by:

a) adding the slurry to the column after the slurry is formed by premixing the stationary phase with a mobile phase; or

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

5. The method of claim 4, wherein all chromatography conditions other than the standing time are identical to the column chromatography performed without a standing time or performed after a slurry is settled for a reduced standing time that is less than the standing time of about 10 minutes or longer.

6. The method of claim 1, wherein the column is under atmospheric pressure during standing time.

7. The method of claim 1, wherein the standing time is between 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.

8. The method of claim 1, wherein the column is an axial compression column.

9. The method of claim 1, wherein the slurry comprises the stationary phase and one or more of water, methanol, ethanol, acetonitrile, ethyl acetate, hexanes, or dichloromethane.

10. The method of claim 9, wherein the slurry comprises methanol.

11. The method of claim 9, 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.

12. The method of claim 9, wherein the stationary phase comprises particles of C18.

13. The method of claim 1, comprising performing elution with a mobile phase comprising one or more of water, methanol, ethanol, acetonitrile, ethyl acetate, hexanes, dichloromethane, or supercritical carbon dioxide.

14. The method of claim 13, wherein the mobile phase comprises methanol.

15. The method of claim 13, wherein the mobile phase comprises an additive selected from one or more of formic acid, ammonium formate, trimethylamine, ammonia, and

ammonium hydroxide.

16. The method of claim 13, wherein the mobile phase constitutes a mobile phase gradient.