system uses packed loops in the interface to prevent the analytes eluted from the SFC column from being forced into the waste line by the CO_2 stream, and water is introduced into the loop to reduce interference of residual CO_2 gas in the second dimension.

Cortes and co-workers (*J. Microcol. Sep.* **1992**, 4:239-244; and U.S. Patent No. 5,139,681) described a 2D LC x SFC system including a sample inlet capillary where volatile solvents from the first dimension LC is eliminated by passage of nitrogen gas leaving a deposit of the eluted analytes, which is then taken up by the CO_2 mobile phase for the second dimension SFC. However, solvent elimination by passage of nitrogen gas is not practical for RPLC which uses aqueous mobile phase.

There remains a need for an efficient chromatography system and methods for separating and analyzing complex samples where it is advantageous to conduct a first dimension RPLC and a second dimension SFC separation.

BRIEF SUMMARY

Disclosed are multi-dimensional chromatography systems and methods for separating and/or analyzing complex mixtures of organic compounds, for example, a two-dimensional reversed-phase liquid chromatography (RPLC) – supercritical fluid chromatography (SFC) system, particularly a RPLC × SFC system including an interface capable of retaining the analytes eluted from the RPLC column while letting the RPLC mobile phase pass through, thus reducing the amount of RPLC solvents carried on to the SFC column.

Disclosed is a chromatography system for separating a sample comprising: (i) a first separation unit comprising: a) a first pump assembly for driving a first mobile phase through the first separation unit, b) a sample injector for introducing a sample to the first separation unit, and c) a reversed-phase liquid chromatography (RPLC) column; (ii) a second separation unit comprising: a) a second pump assembly for driving a second mobile phase through the second separation unit, and b) a supercritical fluid chromatography (SFC) column; and (iii) a first fluidic routing unit comprising a plurality of sample loops, said first fluidic routing unit is connected to the first separation unit and the second separation unit, wherein at least one of the plurality of sample loops comprises a trapping column, said trapping column comprising a stationary phase; and wherein the chromatography system is configured for first separating the sample in the first

separation unit and subsequently introducing at least a portion of the sample eluted from the RPLC column to the second separation unit. In one aspect, provided is a chromatography system for separating a sample comprising: a first separation unit comprising: a) a first pump assembly for driving a first mobile phase through the first separation unit, b) a sample injector for introducing a sample to the first separation unit; and c) a reversed-phase liquid chromatography (RPLC) column; a second separation unit comprising: a) a second pump assembly for driving a second mobile phase through the second separation unit, and b) a supercritical fluid chromatography (SFC) column; and, a first fluidic routing unit comprising a plurality of sample loops, said first fluidic routing unit is connected to the first separation unit and the second separation unit, wherein at least one of the plurality of sample loops comprises a trapping column, said trapping column comprising a stationary phase; and wherein the chromatography system is configured for first separating the sample in the first separation unit and subsequently introducing at least a portion of the sample eluted from the RPLC column of the first separation unit to the second separation unit via the first fluidic routing unit. In some embodiments, the system further comprises detector(s) for the first separation unit and/or the second separation unit. The system may further comprise one or more control devices operably connected to one or more of the system components.

In some embodiments, the 2D RPLC x SFC system comprises a first fluidic routing unit which comprises two sample loops; wherein one of the two sample loops is in fluidic communication with the first separation unit and the other one of the two sample loops is in fluidic communication with the second separation unit. In some embodiments, the first fluidic routing unit comprises three or more sample loops, and wherein one or more of the sample loops is in fluidic isolation from the first separation unit and the second separation unit. In one variation, at least one sample loop that is in fluidic isolation from the first separation unit and the second separation unit comprises a trapping column loaded with a stationary phase material. In some embodiments, the first fluidic routing unit is configured to allow countercurrent elution of analytes retained in a trapping column. In some embodiments, the first fluidic routing unit is configured to allow co-current elution of analytes retained in a trapping column.

In some embodiments, the 2D RPLC x SFC system comprises a second separation unit which comprises one SFC column, and optionally a focus column positioned upstream of the SFC column. In some embodiments, the second separation unit comprises a parallel array of SFC columns, each optionally comprise a focus column positioned upstream of the SFC column.

Further provided are methods of using the chromatography systems described herein. In some embodiments, provided is a method for analyzing a samples (such as a complex sample) using a chromatography system described herein comprising: first separating the complex sample into fractions by RPLC; and further separating the fractions from the RPLC dimension by SFC in the second dimension.

In one embodiment, provided is a method for simultaneous achiral-chiral analysis of a sample comprising a mixture of stereoisomeric components using a chromatography system described herein comprising: separating (or resolving) one or more diastereomeric component(s) of interest in the sample by RPLC in the first dimension, which provides achiral purity of the sample; and

separating (or resolving) the enantiomeric pair(s) of interest by SFC in the second dimension in the same analytical run, which further provides chiral purity of the components in the sample.

In this disclosure, provided is a method for separating a sample by multi-dimensional chromatography (such as 2D RPLC x SFC) comprising the steps of: (i) capturing at least a portion of a sample on a trapping column, said portion is from separating the sample by reversed-phase liquid chromatography (RPLC), said trapping column comprising a stationary phase; and (ii) subjecting the portion of the sample captured on the trapping column to further separation by supercritical fluid chromatography (SFC).

In another aspect, provided is a method for separating a sample comprising the steps of: (i) capturing at least a portion of a sample on a trapping column, said portion is obtained by separating the sample by reversed-phase liquid chromatography (RPLC), said trapping column comprising a stationary phase; and (ii) subjecting the portion of the sample captured on the trapping column to further separation by supercritical fluid chromatography (SFC).

In another aspect, provided is a method for analyzing a sample using a chromatography system as defined herein comprising: separating the sample into a first set of fractions by reversed-phase liquid chromatography (RPLC) on the first separation unit; and further separating one or more of the fractions by supercritical fluid chromatography (SFC) on the second separation unit.

In another aspect, provided is a method for for achiral-chiral analysis of a sample comprising a mixture of stereoisomeric components using a chromatography system as defined herein comprising: separating one or more diastereomeric component(s) of interest in the sample by RPLC on the first separation unit; and separating the enantiomeric pair(s) of interest by SFC on the second separation unit in the same analytical run.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic of an exemplary multidimensional chromatography system **10**.

FIG. **2A** is a schematic of an exemplary first separation unit **20**, interfaced with a first fluidic routing unit **30**.

CLAIMS

1. A chromatography system for separating a sample comprising:

a first separation unit comprising:

a) a first pump assembly for driving a first mobile phase through the first separation unit,

b) a sample injector for introducing a sample to the first separation unit; and

c) a reversed-phase liquid chromatography (RPLC) column;

a second separation unit comprising:

a) a second pump assembly for driving a second mobile phase through the second separation unit, and

b) a supercritical fluid chromatography (SFC) column;

and,

a first fluidic routing unit comprising a plurality of sample loops, said first fluidic routing unit is connected to the first separation unit and the second separation unit,

wherein at least one of the plurality of sample loops comprises a trapping column, said trapping column comprising a stationary phase;

and

wherein the chromatography system is configured for first separating the sample in the first separation unit and subsequently introducing at least a portion of the sample eluted from the RPLC column of the first separation unit to the second separation unit via the first fluidic routing unit.

2. The chromatography system of claim 1, wherein the first fluidic routing unit comprises two sample loops; wherein one of the two sample loops is in fluidic communication with

25. The method of any one of claims 22 to 24, further comprising eluting the portion of the sample captured on the trapping column off the trapping column.

26. The method of any one of claims 22 to 25, further comprising detecting a component of the sample after further separation by SFC.

27. The method of any one of claims 22 to 26, further comprising positioning the trapping column in a flow path of the first mobile phase downstream of the RPLC column for capturing at least a portion of the sample separated by the RPLC column.

28. The method of claim 27, further comprising switching the trapping column carrying the captured portion to a flow path of a second mobile phase for eluting the captured portion off the trapping column.

29. The method of claim 28, wherein the step of positioning the trapping column in the flow path of the first mobile phase or the step of switching the trapping column to the flow path of the second mobile phase is performed in a fluidic routing unit interfacing a fluidic path of the RPLC and a fluidic path of the SFC.

30. The method of any one of claims 22 to 29, further comprising re-capturing at least a portion of the sample eluted off the trapping column on a focus column prior to further separation by SFC.

31. The method of any one of claims 22 to 30, wherein a first mobile phase flows through the trapping column in a first direction, and the portion of the sample captured on the trapping column is eluted off the trapping column by flowing a second mobile phase through the trapping column in a direction opposite to the first direction.

32. The method of any one of claims 22 to 30, wherein a first mobile phase flows through the trapping column in a first direction, and the portion of the sample captured on the trapping column is eluted off the trapping column by flowing a second mobile phase through the trapping column in a direction same as the first direction.

33. The method of claim 22, comprising the steps of:

37. The method of any one of claims 22 to 36, wherein further separation by SFC is performed on a SFC column comprising a normal phase stationary phase.

38. The method of any one of claims 22 to 36, wherein further separation by SFC is performed on a SFC system comprising an array of SFC columns.

39. The method of claim 38, wherein each of the SFC columns independently comprises a normal phase stationary phase.

40. The method of claim 39, further comprising routing the portion of the sample captured on the trapping column to a SFC column for further separation, said SFC column comprises a stationary phase adapted for separating the components in the sample.

41. A method for analyzing a sample using a chromatography system of claim 1 comprising:

separating the sample into a first set of fractions by reversed-phase liquid chromatography (RPLC) on the first separation unit; and

further separating one or more of the fractions by supercritical fluid chromatography (SFC) on the second separation unit.

42. The method of claim 41, wherein separation by RPLC on the first separation unit is based in part on a first characteristic of the sample and separation by SFC on the second separation unit is based in part on a second characteristic of the sample, said second characteristic of the sample is different from the first characteristic of the complex sample.

43. The method of claim 41, wherein the sample comprises a mixture of stereoisomeric components.

44. The method of claim 43, wherein the diastereomeric components are separated into one or more fractions by RPLC on the first separation unit, each said fraction comprising an enantiomeric pair.

45. The method of claim 44, wherein separation by RPLC on the first separation unit is based in part on the hydrophobicity of the sample.

46. The method of claim 44 or 45, wherein the enantiomeric pair is further separated into individual enantiomers by SFC on the second separation unit.

47. The method of claim 46, wherein separation by SFC on the second separation unit is based in part on the chirality of the sample.

48. A method for achiral-chiral analysis of a sample comprising a mixture of stereoisomeric components using a chromatography system of claim 1 comprising:

separating one or more diastereomeric component(s) of interest in the sample by RPLC on the first separation unit; and

separating the enantiomeric pair(s) of interest by SFC on the second separation unit in the same analytical run.

49. The method of claim 48, further comprising determining an achiral purity based on a chromatogram from the RPLC separation and determining a chiral purity based on a chromatogram from the SFC separation.